A STUDY OF SERUM SOLUBLE CD40 LIGAND LEVEL IN PATIENTS WITH ACUTE CORONARY SYNDROME

Dissertation Submitted for

M.D DEGREE BRANCH - XIII

[BIOCHEMISTRY]

DEPARTMENT OF BIOCHEMISTRY

THANJAVURMEDICAL COLLEGE,

THANJAVUR

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY,

CHENNAI

APRIL - 2013

CERTIFICATE

This is to certify that dissertation titled **A STUDY OF SERUM SOLUBLE CD40LIGAND LEVEL IN PATIENTS WITH ACUTE CORONARY SYNDROME** is a bonafide work done by **Dr.M.MANONMANI**, under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College Thanjavur during her post graduate course from 2010 to 2013.

(Dr.C.GUNASEKARAN,M.D.,DCH)
THE DEAN
Thanjavur Medical College
Thanjavur-4

(Dr.N.SASIVATHANAM, M.D.,DGO)
Professor and Head of the Department
Department of Biochemistry
Thanjavur Medical College
Thanjavur-4.

DECLARATION

I, **Dr.M.MANONMANI**, hereby solemnly declare that the dissertation titled**"A STUDY OF SERUM SOLUBLE CD40 LIGAND LEVEL IN PATIENTS WITH ACUTECORONARY SYNDROME"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of Department **Dr.N.SASIVATHANAM,M.D (Bio)., D.G.O**. This dissertation is submitted to Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch-XIII) in Biochemistry.

Place: THANJAVUR

(Dr.M.MANONMANI)

Date:

ACKNOWLEDGEMENT

I am extremely grateful to The **Dean,**Thanjavur Medical college for permitting me to do this dissertation at Thanjavur Medical College, Thanjavur.

I am indebted greatly to my Professor and Head of Department, Department of Biochemistry**, Dr.N.SASIVATHANAM.M.D (Bio)., D.G.O**, who had inspired and guided me in every step of this study.

I express my sincere gratitude to **Dr.G.SENTHILKUMAR,M.D,D.M**, Associate Professor, Department of cardiology for his valuable help.

I express my heartiest thanks to **Dr.K.NIRMALADEVI,M.D(Bio).,D.C.H**, **Dr.P.ELANGO,M.D (Bio**)., Associate Professors, Department of Biochemistry, Thanjavur Medical College for her help and suggestions for performing my study.

I sincerely thank my Assistant Professors **Dr.R.RAJESWARI,M.D (Bio)., D.D**, **Dr.M.RAMADEVI,M.D (Bio).,D.C.H**, Department of Biochemistry for her support during my study.

I owe my thanks to my co-post graduates for their support during the study.

I would like to acknowledge the assistance rendered by Non Medical Assistants and the Technical staffs who helped me to perform the study.

I am greateful to all my parents and volunteers who participated in this study.

I owe my special thanks to my family members for their moral support in conducting the study.

Above all I express my sincere thanks and prayers to the DIVINE FORCE which guides me through out my life towards the best.

CONTENTS

ABBREVIATIONS

IHD- ISCHEMIC HEART DISEASE

CHD- CORONARY HEART DISEASE

DM- DIABETES MELLITUS

HT- HYPER TENSION

HDL-C -HIGH DENSITY LIPOPROTEIN CHOLESTEROL

CAD- CORONARY ARTERY DISEASE

ATP- ADENOSINE TRI PHOSPATE

MI- MYOCARDIAL INFARCTION

ACS- ACUTE CORONARY SYNDROME

STEMI- ST ELEVATION MYOCARDIAL INFARCTION

NSTEMI- NON ST ELEVATION MYOCARDIAL INFARCTION

ECG- ELECTRO CARDIO GRAM

WHO- WORLD HEALTH ORGANIZATION

LDL-C -LOW DENSITY LIPOPROTEIN CHOLESTEROL

AMI- ACUTE MYOCARDIAL INFARCTION

LDH-LACTATE DEHYDROGENASE

AST-ASPARTATE TRANSAMINASE

A STUDY OF SERUM SOLUBLE CD 40 LIGAND LEVEL IN PATIENTS WITH ACUTE CORONARY SYNDROME

ABSTRACT

Introduction:

Coronary heart disease is an impairment of heart function due to inadequate blood flow to the heart. It is the most common cause of death worldwide by 2020. Serum sCD40L is an inflammatory marker. It is released from the platelets as soon as the onset of the chest pain. It is a strong predictor of cardio vascular risk factor.

Aims and Objectives

To estimate Serum sCD40Ligand, Serum CK-MB, Serum Lipid Parameters, Serum LDH, and Serum AST levels in patients with ACS.

Materials and methods

Study group comprised of 50 subjects were admitted in ICCU with ACS (33ST elevation MI, 4 Non ST elevation MI, 13 UA). 50 healthy sex and age matched subjects were taken as control groups. Venous blood samples were collected. Serum Soluble CD40Ligand was estimated by ELISA technique, Serum CK-MB estimated by Immuno-

inhibition method, Serum Lipid profile by enzymatic assay methods. Serum LDH by DKGC method, Serum AST by Modified IFCC method.

Results

Serum sCD40L($p<0.0001$), Serum TC, Serum TGL, Serum LDL-C, Serum VLDL were significantly increased in the study group, significant decrease in Serum HDL-C ($p<0.0001$) in study group compared to control group. By using pearsons correlation there is positive correlation between Serum sCD40L and Serum CK-MB, Serum TC, Serum TGL($p<0.01$) and negative correlation between Serum sCD40L and Serum HDL-C ($p<0.01$).

Conclusion

sCD40L can be used as a marker in individuals with Acute Coronary Artery disease.

Key words: sCD40L, ACS, MI, CK-MB, Lipid Parameters.

INTRODUCTION

Coronary heart disease (syn: ischaemic heart disease)-is defined as cardiac function impairment. There is an inadequate blood flow in the heart compared to its needs and obstructive changes in the coronary circulation (1982)[1].

CHD is a world wide health epidemic. All over the world it is the commonest cause of death. In USA, European countries, Japan, Singapore, Canada etc, this is the first common cause of death. In India it is one in ten common causes of death[2]. Epidemics of CHD in United States, began in the early 1920s, in the 1930s in the Britain, still later in several European countries[1] .

World wide cardiovascular death is about 30 percent. Of which CHD is more than half. CHD has been classified as Acute Coronary Syndrome, Chronic CHD, and sudden death. Clinically in many ways CHD may present, extending from an asymptomatic finding to unexpected cardiac collapse.

Chronic CHD is always secondary to coronary atherosclerosis, leading to coronary blood flow mismatch and adenosine triphosphate homeostasis (imbalance of demand and supply) and coronary ischaemia. Acute coronary

syndrome is a unifying term representing a common end result, acute myocardial ischaemia.

Acute ischaemia is usually caused by atherosclerotic plaque rupture, erosion, fissuring, or a combination with superimposed intracoronary thrombosis and is associated with an increased risk of myonecrosis and cardiac death. It encompasses acute myocardial infarction and unstable angina (resulting in ST elevation or non- ST elevation).

ACS in emergency department should be triaged immediately to an area with defibrillation capability and continuous electrocardiographic monitoring[3]. . Diagnosis of ACS at this earlier stage can prevent morbidity and mortality. Along with ECG changes, proteins in the serum like CK-MB, Cardiac Troponin-I &T, LDH and AST are used to diagnose ACS. However, in the absence of necrosis these markers are not elevated when measured in the first 2-6 hours following an ischemic event.

Serum sCD40 Ligand – is a type II transmembrane glycoprotein .It is structurally related to Tumour Necrosis Factor -alpha. It is expressed within seconds after platelet activation and it has been shown to be proinflammatory

for endothelial cells, which ultimately lead into endothelial dysfunction and atherosclerosis.

Hence in the present study, serum levels of sCD40L were estimated in patients with ACS and the relationship between Serum sCD40L, Serum CK-MB and lipid profile were analyzed.

REVIEW OF LITERATURE

Ischaemic Heart Disease is due to inadequate supply of oxygen and blood flow to the myocardium. There is an imbalance between demand and myocardial oxygen supply, mainly due to epicardial coronary artery atherosclerosis. Because of arterial involvement myocardial perfusion is impaired. There is regional reduction in myocardial blood flow.

Epidemiology:

IHD causes more deaths, disabilities, and economic losses. It occurs mainly in the developed countries than any other illness.

In US IHD is the most life threatening, chronic and serious illness. Incidence of MI is >7 million and of IHD is >13 million and of Angina pectoris is >6 million.

Emergence of IHD is associated with energy rich and a high fat diet, smoking and sedentary life style. In the Western Europe and United States, it occurs more in low socioeconomic group of people, progression of disease can be delayed by primary prevention .

Risk factors for IHD are Obesity, Type 2 Diabetes Mellitus and insulin resistance. In South Asian countries, especially in India, the population subgroups particularly affected by IHD are men.

By 2020, IHD is the most common cause of death worldwide[4,5].

Classification of ischaemic heart diseases:

Ischaemic heart disease divided into two groups:

1. Acute coronary syndrome c
2. Chronic coronary artery disease

Risk factors for Acute Coronary syndrome:

I) Major risk factors:

A) Modifiable:

1) Dyslipidaemia
2) Hypertension
3) Diabetes mellitus
4) Smoking

B) Constitutional:

1) Age
2) Sex
3) Genetic factors
4) Familial and Racial factors

II) Emerging risk factors:

1) Environmental influences
2) Obesity
3) Hormones- Oestrogen deficiency, Oral Contraceptives
4) Physical inactivity
5) Stressful life
6) Homocystinuria
7) Role of alcohol
8) Prothrombotic factors

9) Infections (Chlamydia Pneumonia, Herpes virus, Cytomegalo virus)
10) High C- Reactive protein
11) Other dietary habits
12) Social deprivation

Dyslipidemia:

Chronic dyslipidemia initiates endothelial injury and dysfunction and causes increased permeability. Hypercholesterolemia with increased serum concentration of LDL promotes formation of foam cells[6,7].

Hypertension:

Incidence of atherosclerosis is more with elevated blood pressure.

Hypertension doubles the risk of all forms of cardiovascular diseases. Mechanical injury to the arterial wall is due to increased blood pressure.

Elevation of diastolic blood pressure more than 95 mm of Hg and Systolic blood pressure more than 160 mm of Hg is associated with five times the increased risk of developing IHD than in people with blood pressure within normal range(140/90 mm of Hg or less)[6,8].

Diabetes Mellitus:

In people with type I and Type II Diabetes Mellitus, clinical manifestations of atherosclerosis are far more common and develop at an early age.

The risk of developing IHD is doubled in Type 2 DM. Endothelial dysfunction, increased aggregation of platelets, increased LDL-C, and decreased HDL-C are the causes of increased severity of atherosclerosis.

Glucose intolerance accounts for a major part of the high incidence of ischemic heart disease in certain ethnic groups, (eg): South Asians[9].

Smoking:

In smokers, the extent and severity of atherosclerosis are much greater than in non-smokers. Cigarette smoking is associated high risk of atherosclerotic IHD and sudden cardiac death.

Men who smoke a pack of cigarettes per day are 3-5 times more risk to die of IHD than non-smokers.

The increased risk of atherosclerosis in smokers could be attributed to the following factors:

- Altered serum lipids (Elevated LDL-C and reduced HDL-C levels).
- Accumulation of carbon monoxide in the blood produces carboxy haemoglobin which in turn favours atherosclerosis by producing hypoxia in the arterial wall.
- Increased platelet aggregation and vasospasm produced by nicotine- reduces myocardial oxygen supply.
- Smoking causes release of catecholamines which increases heart rate and blood pressure and myocardial oxygen demand[10].

Constitutional risk factors:

Age:

Atherosclerosis is an age related disease. In childhood, early lesions of atherosclerosis may be present whereas clinically significant lesions are found in the fourth decade. Beyond fourth decade, fully developed atheromatous plaques appear.

Sex:

Premenopausal women have lower rates of disease than men. In females it is due to high level of oestrogen and high density lipoprotein, both of which have anti-atherogenic influence. After the menopause this sex difference disappears. The incidence and severity of atherosclerosis are more in men than women and changes appear a decade earlier in men (>45 years) than women (>55 years).

The prevalence of atherosclerotic IHD is about three times higher in men in 4^{th} decade than women and the difference slowly declines with age but remains higher at all ages in men[11,12].

Genetic factors:

There is genetic derangement of lipoprotein metabolism. It leads to high serum lipid levels and familial hypercholesterolemia.

The impact of genetic risk of CHD in twins-a monozygotic twins are eight fold increased risk, and a dizygotic twins are fourfold increased risk of dying from CHD compared to general population[6,10].

Familial and Racial factors:

Familial predisposition to atherosclerotic vascular disease, mainly depends on combination of genetic, environmental and lifestyle factors. The familial predisposition to atherosclerosis may be related to other risk factors like DM, HT, Hyperlipoproteinemia.

A Cases can be diagnosed when clinical problems occur at a relatively young age (<50 years for men and <55 years for women) in the first degree relatives. Racial differences too exist; Blacks have generally less severe atherosclerosis than Whites[8,10].

Emerging risk factors:

1. **Enviromental influences:**

Higher incidence of atherosclerosis in developed countries and low incidence in underdeveloped countries suggest the role of environmental influences.

2. Obesity:

If the person is overweight by 20% or more, it is associated with increased risk. Atherosclerosis is more likely related to android or upper body obesity than to general obesity[7].

3. Hormones:

Oral contraceptive administration or endogenous oestrogen deficiency (eg: in post menopausal women) has an increased risk of developing Myocardial Infarction or Stroke.

4. Physical inactivity:

The risk of CHD is doubled by physical inactivity. Regular exercise has been shown to have a protective effect by increasing in serum HDL-C levels, lowering the blood pressure and development of collateral vessels.

5. Stressful life style:

This is termed as Type A behavior pattern and is characterized by aggressiveness , competitive drive, ambitiousness and sense of urgency. It is associated with enhanced risk of IHD when compared with Type B behavior of relaxed and happy –go –lucky type.

6. Homocysteinemia:

It is an uncommon inborn error of metabolism, which can present with early atherosclerosis and CHD[13].

7. Alcohol:

Excess alcohol consumption is associated with HT and Cerebrovascular disease[14].

8. Prothrombotic factors:

Platelet activation and elevated fibrinogen level are associated with increased risk of Coronary thrombosis which is the gravest complication of atherosclerosis. Antiphospholipid antibodies are associated with recurrent arterial thrombosis[10,15,16].

9. Role of infections:

Chlamydia Pneumonia and viruses such as Herpes virus and Cytomegalovirus have been found in coronary atherosclerotic lesions. In combination with some other factors they cause inflammation and atherosclerosis[17,18,51].

10. C-Reactive protein:

It is an acute phase reactant and it correlates with the risk of developing atherosclerosis[9,16].

11. D**ietary products:**

Diets deficient in vegetables, fresh fruits and polyunsaturated fatty acids are associated with increased cardiovascular risk. Mediterranian diet has been shown to reduce cardiovascular events. Dietary supplements such as vitamins C, E, Beta-Carotene, Folate, and Fish oils reduce cardiovascular events.

12. Social deprivation:

Health inequalities also have a major influence on cardio vascular disease[9].

Clinical Types of IHD:

Patients with IHD can be divided into two groups

1) Chronic coronary artery disease
2) Acute Coronary Syndrome

Chronic Coronary Artery Disease:

It is always secondary to coronary atherosclerosis, leading to mismatch of coronary blood flow and ATP homeostasis (imbalance of supply and demand) and a stable pattern of coronary ischemia. The clinical pattern includes stable angina pectoris and Myocardial Infarction.

Angina:

Blood flow to the heart muscle does not provide an adequate supply of oxygen for its requirements. As a result, cardiac muscle becomes ischemic and chest pain occurs.

Pain is substernal, central crushing in nature and may radiate to arms, neck or jaw[17].

Angina may be of different types:

1) **Stable Angina:**

It is due to vasoconstriction which can be evoked by intense sympathetic stimulation. Example: severe stress or severe cold or by narrowing of a coronary artery due to atheroma[18].

2) **Variant Angina (Prinzmetals Angina):**

It is caused by coronary vasospasm. In this type angina occurs at rest with reversible ST segment elevation without enzymatic evidence of MI[19].

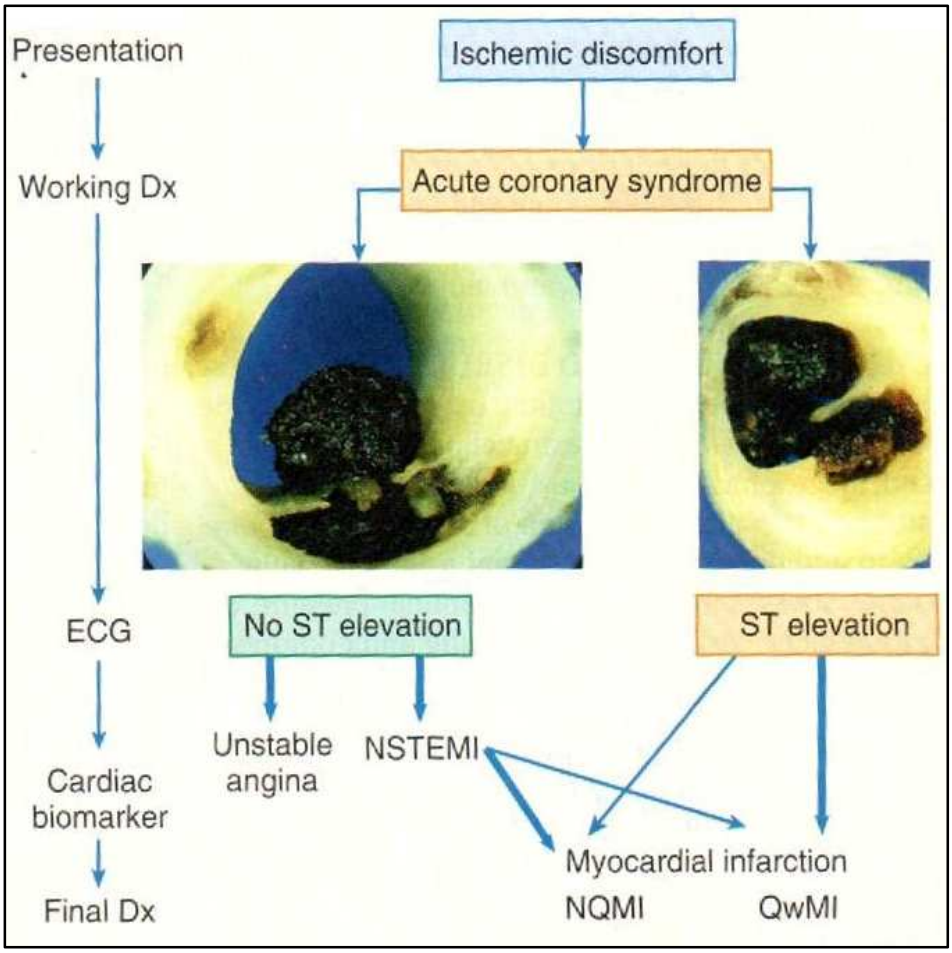
3)Heberdens Angina:

Myocardial ischemia is induced by increased myocardial contraction acting in the face of the relatively constant coronary blood flow prescribed by structural coronary stenosis produced by atherosclerosis[20].

4)Other Anginal Types:

1. Nocturnal Angina: Angina occurs during sleep at night due to coronary ostial stenosis.
2. Angina Decubitus: Angina occurs in recumbent position
3. Stable exertional Angina: Angina occurs predictably at a certain level of exertion.
4. Unfamiliar tasks: Angina may also be precipitated by heavy meal, exposure to cold etc.

MANIFESTATIONS OF ACUTE CORONARY SYNDROME



Acute Coronary Syndrome:

ACS is a unifying term representing a common end result, acute myocardial ischemia. It is caused by rupture, erosion or fissuring of an atherosclerotic plaque which can occur alone or in combination with intracoronary thrombosis. This is associated with an increased risk of myocardial necrosis[21].

It encompasses ischemia with minimal myocardial damage that is unstable angina and Acute Myocardial Infarction[22,23].

Acute Myocardial Infarction may be

1) STEMI (ST elevation Myocardial Infarction)

2) NSTEMI (Non ST elevation Myocardial Infarction)

Recognizing a patient with ACS is important because the diagnosis triggers both triage and management. ACS in the emergency department should be triaged immediately to an area with continuous electrocardiographic monitoring and defibrillation capability.

An ECG should be obtained and accurately interpreted within 10 minutes. Patients with suspected ACS should be managed immediately with antiplatelet and anticoagulant therapies and considerable for immediate revascularization mechanically or pharmacologically if new ST elevation is noted. ACS is a life threatening condition.

Proper initial triage of patients suspected to have coronary ischemia should eventually identify patients as having

(1) Acute Coronary Syndrome.

(2) Non-ACS cardiovascular conditions such as Myocarditis, Myo pericarditis and also Cardiomyopathy related to Stress, Aortic Dissection or Pulmonary Embolism.

(3) A non-cardiac cause of chest pain such as gastro oesophageal reflux.

(4) A non-cardiac condition such as Sepsis.

ACS patients with new evidence of ST elevation on ECG are labeled as having STEMI and should be considered for immediate reperfusion therapy by percutaneous coronary intervention or thrombolytics.

NSTEMI patients present without ST elevation and have evidence of myonecrosis. Unstable Angina patients do not have any evidence of myonecrosis.

Unstable Angina:

Unstable Angina (or) Cresendo Angina (or) Preinfarction Angina: It is defined as rest pain without a recent myocardial infarct (Braunwald class IIIB) and no evidence of major myocardial necrosis as reflected by raised serum Creatine Kinase MB. Transient ST-T segment depression and T wave inversion are often present in this group of patients[24].

Fissuring of plaques causes sudden onset of angina which increases in frequency and severity. There is subsequent total thrombotic occlusion of the vessel. Unstable angina will progress to MI or death may occur due to secondary development of a ventricular arrhythmia[25,26].

Myocardial Infarction:

Irreversible necrosis of part of the heart muscle is almost always due to Coronary atherosclerosis. ECG abnormalities are

1) Inverted T wave due to ischemia

2) ST abnormalities due to muscle injury

3) Pathological Q wave due to muscle death. If coronary blood flow is interrupted, myocyte necrosis occurs for an extended period of time.

Pathologically – There are two types of MI

1. Subendocardial MI

2. Transmural MI

In MI plaque, there is progression, disruption, subsequent clot formation. The thrombus is quite stable and occludes the vessel for a prolonged period, ultimately causing progression of myocardial ischemia to myocyte necrosis and death[27].

ST segment elevation MI:

In this condition, a completely occlusive thrombus results in total cessation of coronary blood flow in the territory of the occluded artery. It is the most stable form of ACS. Full or nearly full thickness necrosis of the ventricular wall supplied by the occluded artery leads to formation of new Q wave .

The actual diagnosis of STEMI is not completely based on ECG findings. The classic WHO criteria for an acute myocardial infarction requires the two of the following three elements to be present:

(1) A history suggestive of coronary ischemia for a prolonged period of time (>30 minutes)

(2) A rise and fall in serum cardiac markers with myonecrosis.

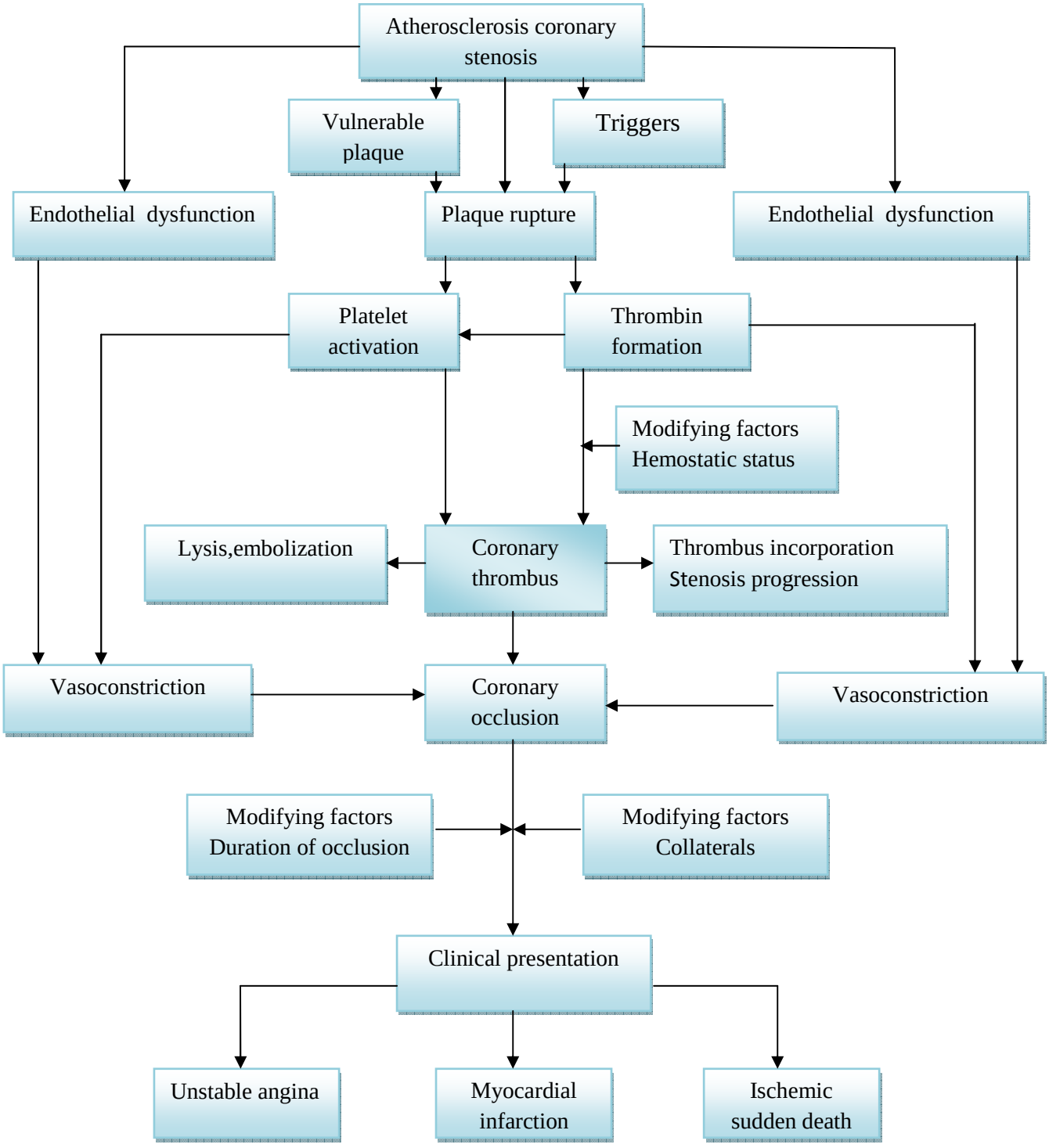
(3) Evolutionary changes on serial ECGs suggestive of MI

(3) A rise and fall in serum cardiac markers with myonecrosis[(3)].

Non ST segment elevation MI:

Patients with clinical features of unstable angina develop evidence of myocardial necrosis which is diagnosed by elevated cardiac biomarkers. The ECG show no ST elevation, T wave inversion, and ST depression exceeding 2 mm.

PATHOPHYSIOLOGIC EVENTS IN ACUTE CORONARY SYNDROME



PATHOPHYSIOLOGY OF ACUTE CORONARY SYNDROME:

Rather than the degree of stenosis, atherosclerotic plaque composition and non stenotic lesions are more frequently implicated in ACS. Non occlusive plaques become complicated by thrombus and progress rapidly to total occlusion.

ACS is due to five main causes:

1. Plaque rupture with acute thrombosis by formation of blood clot that gradually occludes the lumen is the pivotal event in atherothrombotic process.
2. Progressive increase of plaque volume leads to mechanical obstruction.
3. Inflammation by growth factors and enzymes, local and systemic production of mediators of cytokines activate the plaque and disrupt the fibrous cap.
4. Coronary vasoconstriction occurs due to dynamic obstruction influenced by endothelial dysfunction.
5. Plaque embolization occurs at distant level from atherothrombotic coronary occlusion[(28)].

In most cases of ACS, sudden luminal thrombosis is a consequence of either plaque rupture, plaque erosion, or a calcified nodule.

Prolonged chest pain at rest often results from fissuring or rupturing of the atherosclerotic plaque leading to thrombus formation.

Plaques are prone to rupture which contain large amounts of extracellular cholesterol covered by a thin cap of fibrotic tissue. The immediate

site of plaque rupture is marked by an inflammatory process (macrophage rich area).

The inflammation plays a role in destabilizing the fibrous cap tissue. Factors predisposing to plaque rupture are circulating immune complexes, nicotine, hyperlipidemia, high angiotensin level, elevated acetoacetic acid levels in diabetes. The junction between the normal and atherosclerotic segments of an eccentric stenosis is the site for rupture of the fibrous cap[29,30].

Atherosclerosis:

It is a Greek word; Sclerosis means hardening. Coronary artery disease and MI are the number one killers in the world .In India 20% of deaths are due to CAD and Myocardial ischaemia.

It is estimated that by the year 2020, it will account for 33% of all deaths[31]. Atherosclerotic lesions were graded according to the types of lesion by the American College of Cardiology/American Heart Association classification.

Type A lesions are defined as discrete lesions (<10 mm in length).

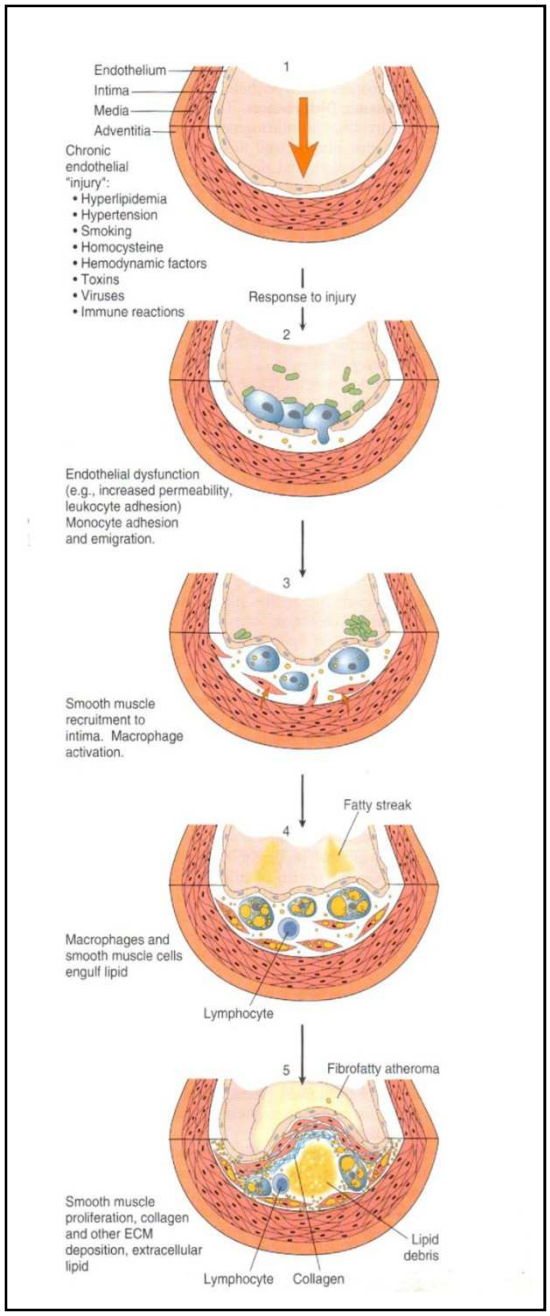
Type B lesions are tubular lesions (10-20 mm in length)

Type C lesions are diffuse lesions (> 2 cm in length)[24].

Atherosclerosis represents a chronic inflammatory response to vascular injury and activate endothelium and promote lipoprotein infiltration, modification and retention combined with inflammatory cell entry, retention and activation[32].

Atherosclerosis can affect any artery in the body. In the heart, it may cause angina, MI and sudden death. In the brain, it may cause stroke and transient ischemic attack. In the limbs, it may cause claudication and critical limb ischemia[33].

PATHOGENESIS OF ATHEROSCLEROSIS



PATHOPHYSIOLOGY OF ATHEROSCLEROSIS:

Endothelial injury is the initiating event in atherosclerosis. Endothelial injury is caused by hypertension, hypercholesterolemia, local hemodynamic abnormalities, cigarette smoking, hyperhomocysteinemia, increased LDL-C , c-reactive protein, increased fibrinogen, insulin resistance ,oxidative stress, infections like Herpes virus or Chlamydia pneumonia, periodontal diseases and combination of these factors[34,35].

Endothelial dysfunction is due to the accumulation of macrophages. These are derived from lipids and circulating monocytes (predominantly LDL-C). At the site of vascular injury, oxidation of low density lipoproteins and their ingestion by macrophages produces foam cells. The foam cells aggregate to form the fatty streak, the earliest visible lesion of atherosclerosis.

The atherosclerosis is a complex disease involving increased pro-oxidant stress, inflammatory fibro-proliferative and angiogenic responses combined with smooth muscle cell proliferation which results in plaque formation[36].

Theories of atherogenesis:

Atherogenesis is the major cause of cardiovascular disease, due to the formation of atheroma which leads to arterial hardening. Several theories have been put forward.

The response to injury hypothesis of Ross:

Arterial vessel wall (Endothelial) injury is the main hypothesis and interaction of platelets with damaged vessel wall.

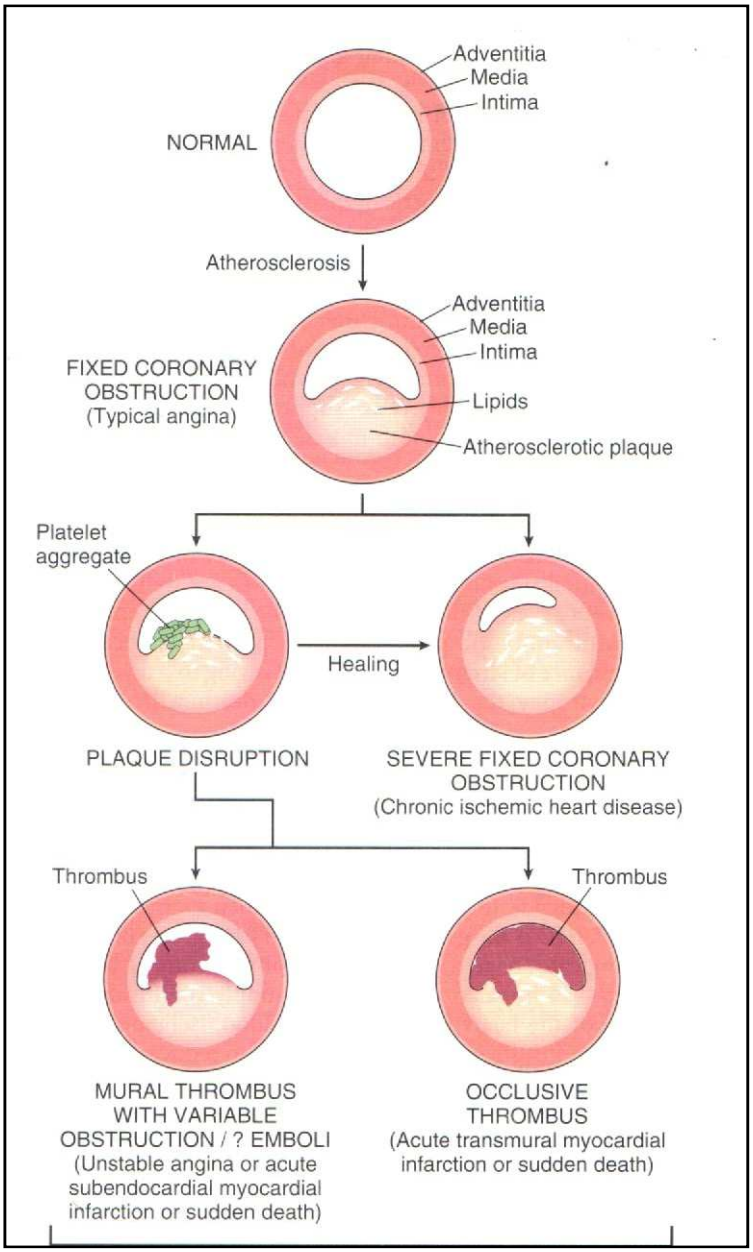
Endothelial injury due to a number of different risk factors, which include viral infection, hyperlipidemia, and smoking[(11)].

The lipid oxidation hypothesis of Steinberg and Collegues:

This hypothesis suggests that mechanism of endothelial injury and formation of macrophage derived foam cells that are characteristic of the early lesions of atherosclerosis.

Leucocytes adhesion to endothelium and subsequent accumulation of T lymphocytes and monocytes in subendothelial space. Within the arterial wall the monocytes are converted to lipid laden foam cells and form macroscopically evident fatty streak lesion.

PROGRESSION OF CORONARY LESIONS LEADING TO ACS



Course of atherosclerosis:

Accumulation of low density lipoprotein particles in the intima is the initial step leading to fatty streaks.

Fatty streaks are formed when inflammatory cells, predominantly monocytes binds to endothelial cell receptors and migrate into the intima. They then develop into lipid laden macrophages or foam cells by taking up oxidized LDL-C that are universally present in atheromatous plaques[37,38,39].

Extra cellular lipid pools appear in the intimal space when these foam cells die and release their contents.

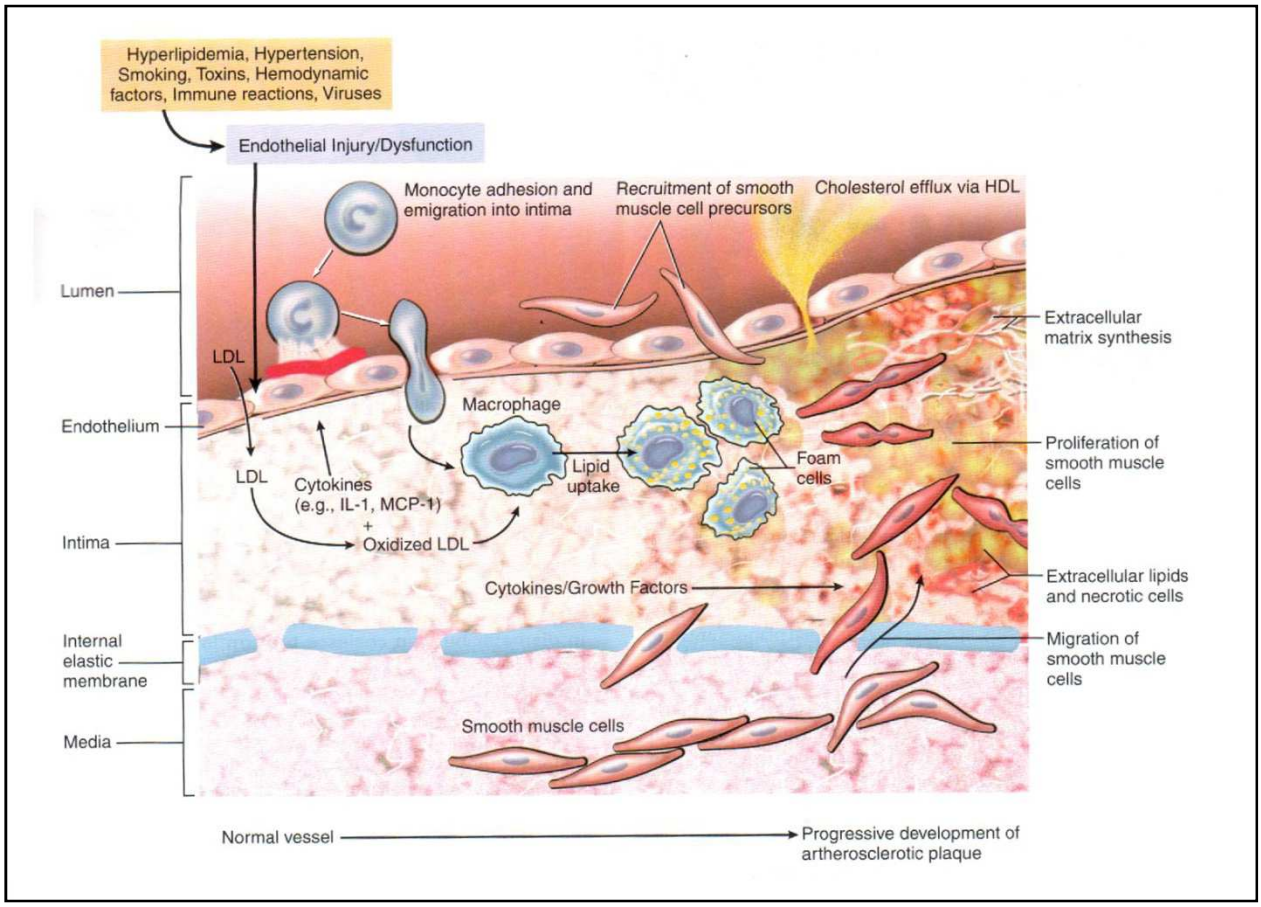
Activated macrophages produces growth factors and cytokines. There is migration of smooth muscle cells into the intima of the arterial wall from the media. Macrophages also stimulates smooth muscle cell proliferation. Oxidized LDL can induce production of Vascular Cell Adhesion Molecule (VCAM-1) -1,Intra Cellular Adhesion Molecule-1(ICAM-1) which can lead onto increase in size of the plaque[40].

Cytokines such as Tumour Necrosis Factor-alpha, interleukin, interferon –gamma, platelet derived growth factor and matrix metalloproteinases are released by activated macrophages. They cause degradation of collagen cross-struts within the plaque and senescence of the intimal smooth muscle cells overlying the plaque[41].

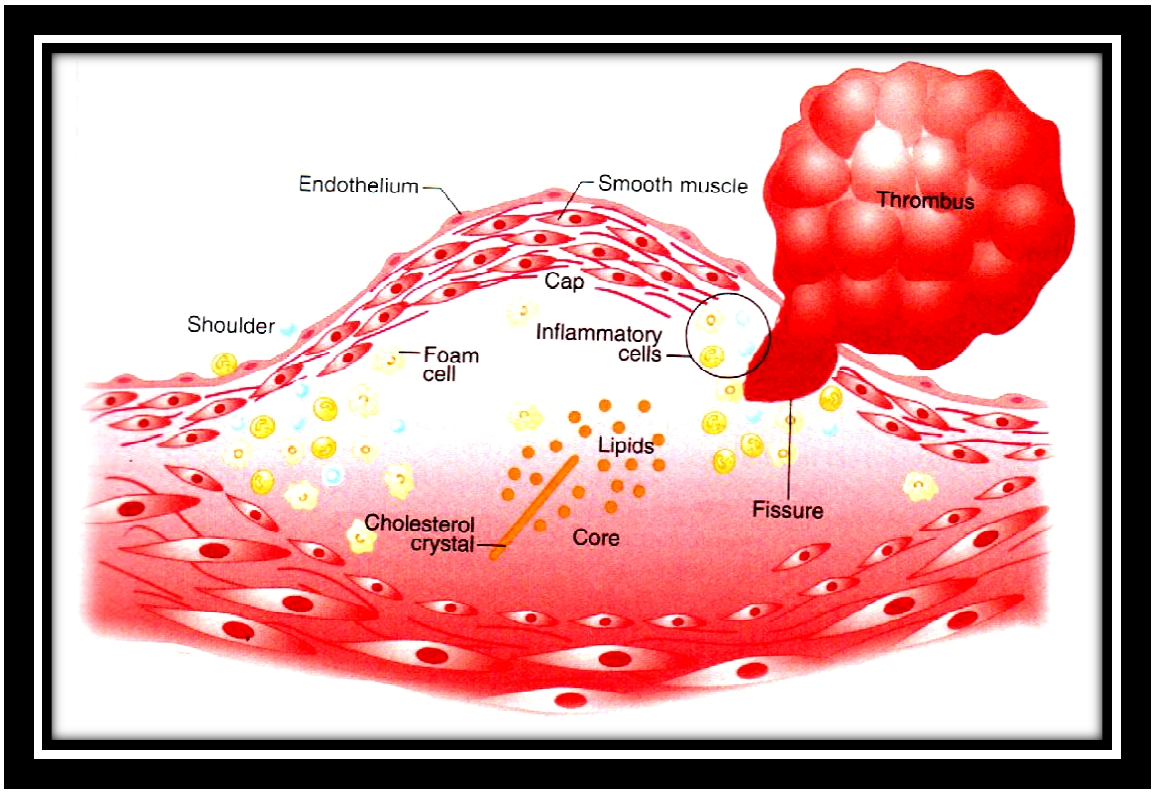
This results in thinning of the protective fibrous cap and thereby causing the lesion vulnerable to mechanical stress. This in turn causes fissuring, erosion or rupture of the surface of the plaque.

If there is any breach in the integrity, the contents of the plaque will be exposed into the blood. Thus will trigger platelet aggregation and thrombosis and extend into the arterial lumen[42,43,44].

PROGRESSIVE DEVELOPMENT OF ATHEROSCLEROTIC PLAQUE



PLAQUE RUPTURE AND ATHEROTHROMBOSIS



Acute changes in plaque fall into three general categories:

1) Rupture/fissuring –exposing highly thrombogenic plaque constituents and subsequent thrombosis. Such thrombosis can partially or completely occlude the lumen and lead to downstream ischemia.

2) Erosion/ulceration exposing the thrombogenic sub-endothelial basement membrane to blood.

3) Hemorrhage into the atheroma expanding its volume. Rupture of the overlying fibrous cap or the thin walled vessels in the areas of neovascularization, can cause intra plaque hemorrhage. A contained hematoma may expand the plaque or induce rupture of the plaque.

Atheroembolism- Plaque rupture can discharge atherosclerotic debris into the blood stream, producing micro emboli[41].

Role of sCD40ligand in Atherogenesis:

Atherogenesis is due to CD40/CD40L interaction on the endothelial cell surface. This results in endothelial and smooth muscle cell activation and subsequent expression of adhesion molecules[51].

CD40L promotes interactions of dentritic cells and T-lymphocytes within the vessel wall. Mapping of activated dentritic cells in coronary

atheroma and human carotid artery showed that CD40L affects dentritic cells and regulate T-cell infiltration, which in turn all required for formation of unstable coronary atheroma[45].

sCD40L activates specific redox sensitive pathways and thereby has an unfavorable effect on endothelium dependent relaxation and vascular redox state.CD40L increases vascular oxygen production by destabilizing the messenger ribonucleic acid of endothelial NO synthase[46].

Therefore pro-inflammatory cytokines, reduced NO bioavailability and over expression of adhesion molecules promote requirement of leucocytes and migration into the media of vessel wall, thereby formation of atheroma.

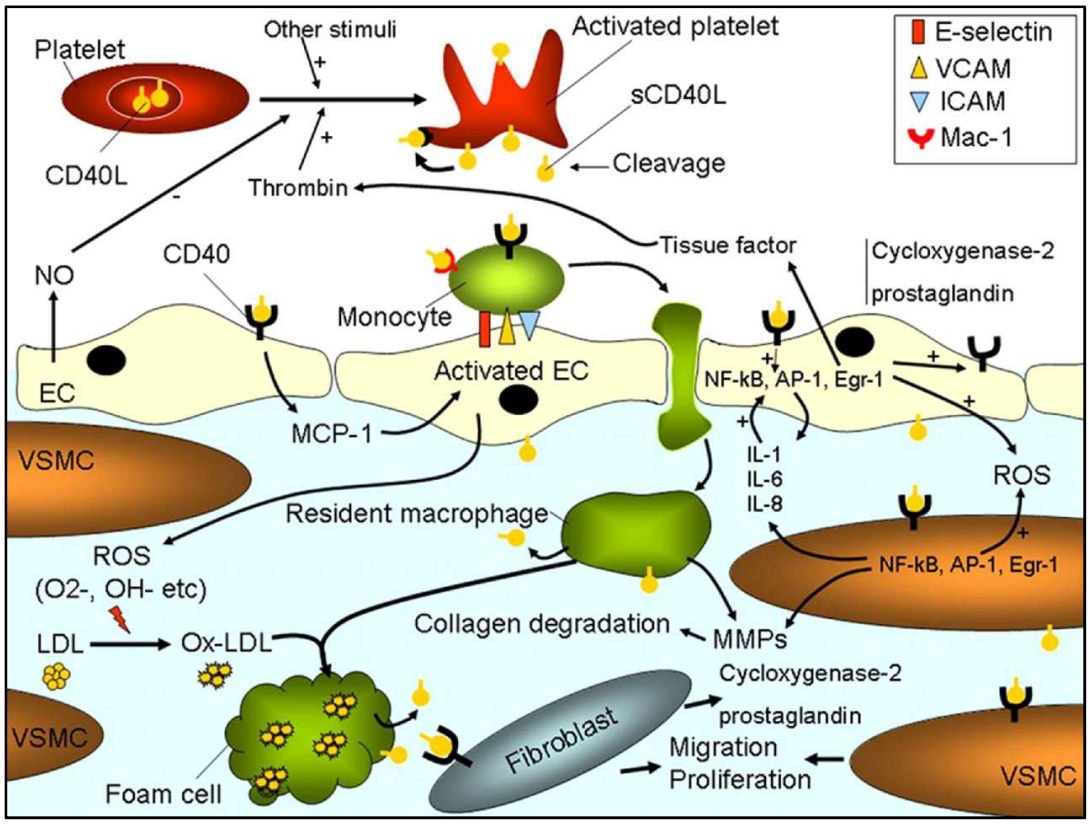
Interaction between CD40L and CD40 also affect the release of adipokines from adipocytes contributing to atherogenesis[47].

CD40L is expressed by activated platelets. It is then cleaved and released and sCD40L binds to circulating monocytes through monocyte / macrophage integrin MAC-1 and CD40Lreceptor and thereby promote adhesion of circulating monocytes to the vascular endothelium[48].

sCD40L also binds to endothelial cell surfaces CD40Lreceptor and activates it. This triggers the over expression of transcriptional factors such as Activator Protein-1(AP-1), nuclear factor kappa B and others to express adhesion molecules such as Intracellular Adhesion Molecule-1(ICAM-1),

E-Selectin and Vascular Adhesion Molecule-1(VCAM-1) on the surface of Endothelial cells[49].

CD40/CD40L AND ATHEROTHROMBOSIS



Adhesion and transmigration of monocytes to subendothelial space is mediated by these molecules. Smooth Muscle Cells and activated endothelial cells also produce proinflammatory molecules such as interleukins and Monocyte Chemoattractant Protein-1(MCP-1). These molecules over express Reactive Oxygen Species (ROS) generating enzymes and also release prothrombotic mediators like tissue factor[50].

The ROS oxidize LDL to oxidized LDL (Ox-LDL) which is taken up by activated macrophages which are turned into foam cells[51,52,53]. sCD40L also activates fibroblasts and smooth muscle cells. They are proliferated and

migrate to further induce atherogenesis. sCD40L regulates the development and stability of thrombus in ACS. This stability is maintained by the interaction between the lysine-arginine glutamic acid domain of the sCD40L and platelet α II b/β3 receptor[54].

CD40/CD40L interactions induce the expression of matrix metalloproteinases that degrades thin fibrous cap of atheromatous plaques and interstitial collagen, leading to plaque instability and rupture[47].

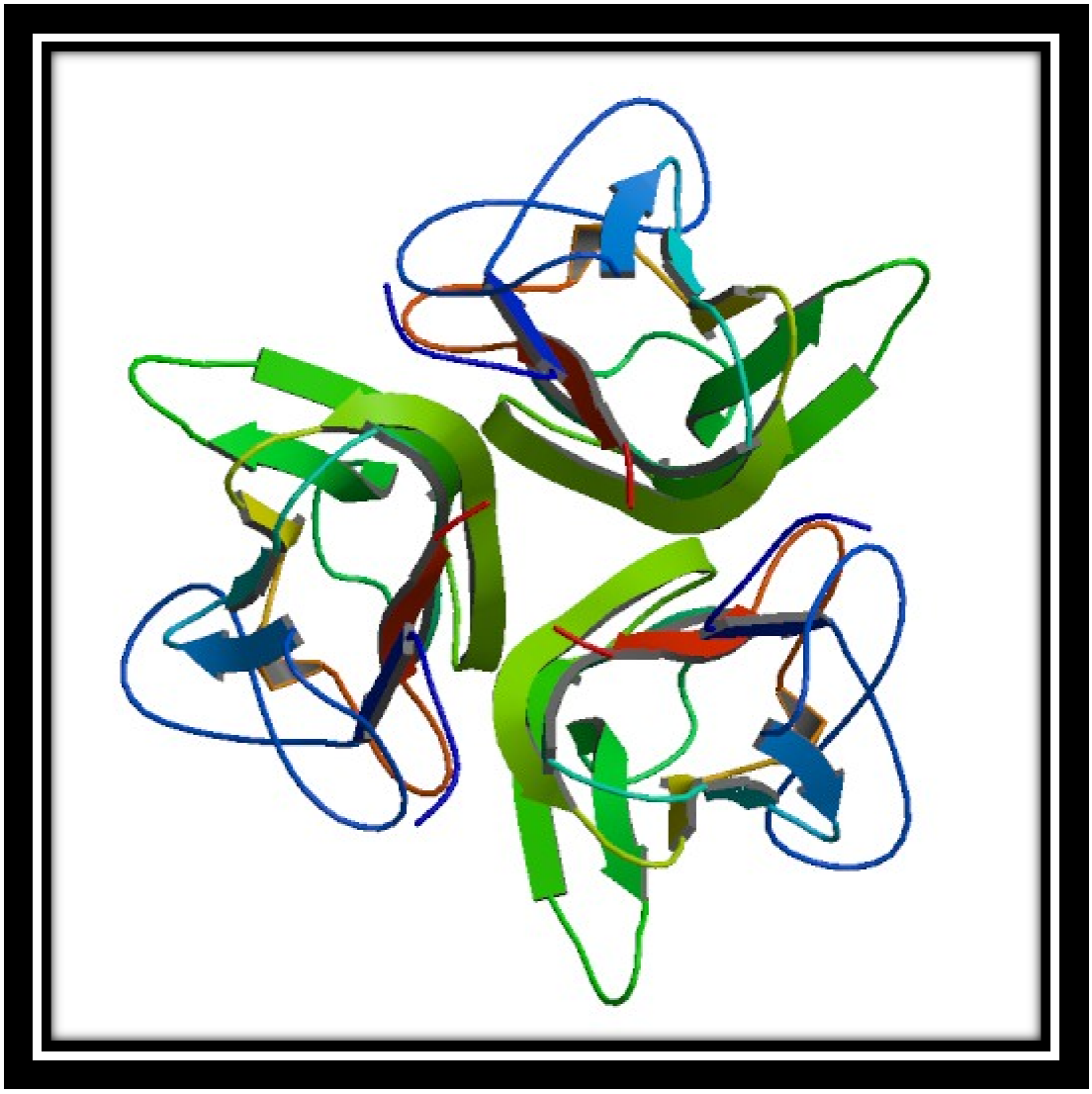
Circulating sCD40L levels are higher in AMI and Unstable angina, compared with healthy persons. Gradual increase in sCD40L level takes place with ACS progression[55,56].

Platelets are the main source of sCD40L, being responsible for 95% of the circulating sCD40L levels[57].

Carriers of 807 T polymorphism on the GP Ia gene which regulates surface density of the receptor, express higher sCD40L levels during the acute phase of MI[58].

Compared to healthy individuals plasma sCD40L is higher in patients with stable angina[59].

STRUCTURE OF SCD40 LIGAND



Soluble CD40Ligand ::

Soluble CD40 ligand is a homotrimeric Type II transmembrane glycoprotein that is related structurally to Tumour Necrosis Factor-alpha[80]. It was first identified on activated platelets and on CD4+cells.Tumour Necrosis Factor homology domain is present in its carboxy terminus which is required for binding to its receptor CD40.

Platelet activation expresses CD40L on platelet membranes. sCD40L, an 18 kDa soluble fragment is produced by subsequent cleavage by metalloproteases. sCD40L binds to CD40 receptor in the endothelial membrane and lead into inflammatory process amplification.

Increased serum sCD40L concentration due to platelet activation has been associated with acute and stable coronary artery disease, chronic inflammatory bowel disease and hypercholesterolemia. Platelet activation is important for thrombus formation which precipitates most of the unstable coronary syndromes[60,61,62,63].

sCD40L plays a crucial role in the pathogenesis of atherosclerosis and coronary artery disease and has been shown to be a promising clinical biomarker of atherothrombotic risk. Soluble form of CD40L and CD40 acts as a mediator between vascular endothelium, platelets and other cell types[50,64,65].

Elevated concentrations of sCD40L causes increased expression of CD40 receptor in human coronary artery endothelial cells. This process is

mediated by extra cellular signal related kinase (ERK)1/2 .Amplification of sCD40L levels produces increased platelet activation and endothelial dysfunction in coronary endothelium[66].

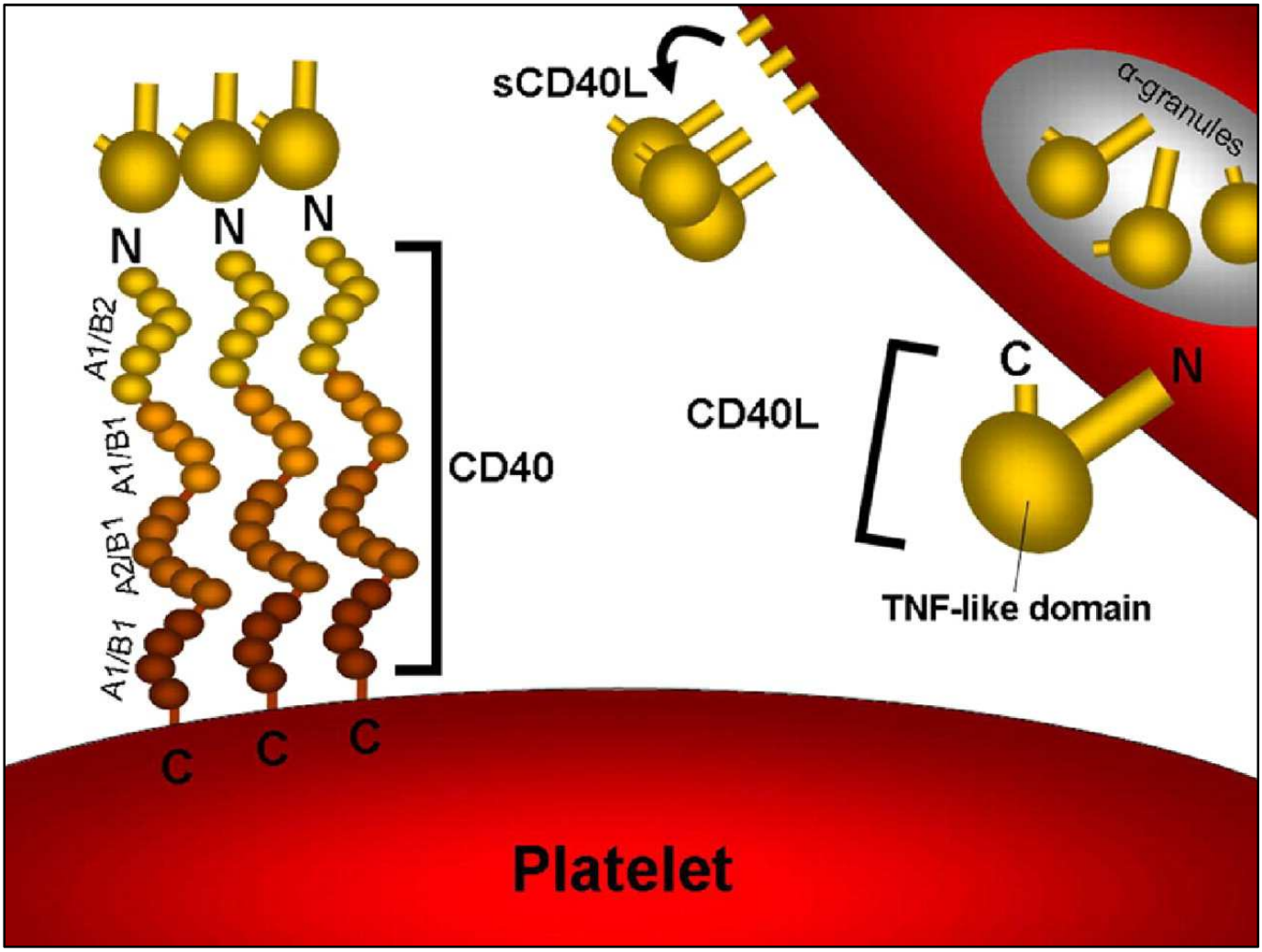
sCD40L contributes to atherosclerotic plaque progression by inducing the expression of chemokines, cytokines, matrix metalloproteinases, growth factors and procoagulant factors in various atheroma associated cell types[67,68].

In vivo platelet activation can be sensitively marked by monocyte-platelet aggregates than P-selectin. Even before the onset of myocardial necrosis, markers of platelet activation can identify the disease activity. sCD40L plays an important role in plaque stabilization and disease progression[57].

sCD40L promotes coagulation by induction of tissue factor expression on monocytes and endothelial cells and is proinflammatory for endothelial cells. Compared to healthy individuals and patients with stable angina, those with unstable angina have been shown to have sCD40L concentrations. This is because of sCD40L release from activated T lymphocytes or platelets[69].

CD40-CD40L system has been implicated in the pathophysioplogy of various chronic inflammatory diseases[50,70,71].

STRUCTURE OF CD40 RECEPTOR, CD40L AND SCD40L



Structure of CD40-CD40L and regulation of its expression:

CD40 is a Type I transmembrane protein and a member of TNF super family[66,72]. Its gene is located in chromosome 20(q12-13.2). CD40 exists as a dimer which trimerizes after binding of CD40L. It exists on cell surface as a constitutional trimer complex, which is crucial for its activation. CD40 has a cysteine rich (consisting of 20 residues) extracellular region.

CD40 protein consist of 4 cysteine rich domains, each domain is divided into two cysteine molecules(A1,B1,A2,B2).The CD40L is a Type II transmembrane protein with an external carboxy terminus and an intracellular amino terminus.

CD40 L gene:

A human sCD40L cDNA has been isolated by screening stimulated human blood T-cell libraries with the murine CD40L probe[73]. The cDNA for human sCD40L encodes a polypeptide of 261 amino acids which consists of a 22 AA cytoplasmic domain, a 24AA transmembrane domain and a 215 AA extra cellular domain with five cysteines.

The gene for human sCD40L is located in the X chromosome at xq26.3-xq27.1 position.

The human sCD40L gene consists of five exons and spans 12-13 kb of chromosomal DNA .The first exon codes for small portion of the extracellular

region and whole of the intracellular transmembrane region. Extra cellular domain is coded by the exons II-IV . This sequence also includes 2000bp approximately of 5 promoter sequences.

sCD40L mRNA has been detected in activated CD4+, CD8+, gamma delta T- cells. It has been detected in other cell types such as dentritic cells, monocytes basophils, mast cells eosinophils and B cells[74].

CD40L protein:

Initial studies revealed a molecular mass of 39 kDa for CD40L, hence called gp39. But the molecular mass of CD40L on most cell types is 32-33 kDa which suggests that some post translational modification take place.

A single N-linked glycosylation site (human Asparagine 240) has been conserved in the extracellular region of human sCD40L. Comparison of human sCD40L (Swiss prot NO:P29965) and mouse sCD40L (Swiss prot NO:P27548) show that there is 78% AA identity between both sequences. There is 75% identity in the extracellular domain , 96% identity between the transmembrane region and 81% between the cytoplasmic domain[75].

sCD40L is produced as a type II transmembrane protein, it may be expressed on the cell surface. In addition to the 33 kDa form, the molecule is associated with two shorter versions of the protein of 31 and or /18 kDa.

Regulation of sCD40L expression:

The expression of sCD40L on activated T-cells is transient and tightly regulated sCD40L expression can be seen on subpopulation of CD4+CD5+ROT cells, as early as 5-15 minutes after anti CD3 activation.

Expression occurs early (1-2 hours) after activation , is maximal after (6-8 hours) and is followed by a gradual loss.

CD40-CD40L inter action leads to

1) release of sCD40L which binds to CD40L
2) receptor mediated endocytosis of CD40L and lysosomal degradation.

3) sCD40L is cleaved proteolytically.

4) down regulation of CD40L mRNA

Thrombin activation of human platelets resulted in CD40L surface expression within one minute. Increased serum levels of sCD40L has been also reported in various collagen diseases such as Systemic Lupus Erythematosus, Systemic Sclerosis and Rheumatoid Arthritis. Autoantibody to CD40L has been demonstrated in SLE[(73).]

A wide range of platelet activators such as thrombin and thrombin receptor agonists like phorbal myristate, collagen stimulate the platelets to express CD40L. Intra cellular calcium concentration and protein kinase C activation regulate CD40L expression.

Nitric oxide signaling also partly regulates the expression of CD40L by platelets. Inhibition of NO synthase in humans induces platelet activation which in turn cause CD40 adipocyte over expression and thereby cause atherogenesis.

Transient expression of CD40L gives the antigen activated T cell , a brief opportunity to deliver helper signals to interacting macrophages, B cells or dentritic cells.CD40L expression is upregulated by CD28, cytokines IL-12,IL-15,IL-2, whereas CD40L expression is downregulated by IL-10,IL-4[(76)].

AIMS AND OBJECTIVES

1. To estimate Serum sCD40Llevel in patients with Acute Coronary Syndrome.
2. To correlate the levels of Serum sCD40L with Serum CK-MB which is an effective marker of Acute Coronary Syndrome.
3. To correlate the levels of Serum sCD40L with Lipid profile.

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College Hospital after getting the approval from the ethical committee. In the present study the age group of both study and control group ranged from 35-68 years , males and females were included and informed consent obtained from them.

50 subjects (30 males and 20 females) who were admitted in ICCU of Thanjavur Medical College Hospital with Acute Coronary Syndrome and with clinical findings suggestive of STEMI (n=33), NSTEMI (n=4) and unstable angina (n=13) were included in the study group. 50 sex and age matched , healthy individuals were taken as control groups.

INCLUSION CRITERIA

1. Patients admitted with complaint of chest pain within 6 hours of onset, chest pain lasting >30 minutes.
2. Electrocardiographic findings showing abnormal ST-T wave changes (ST segment elevation > 1mm in two or more contiguous chest leads or depression or deep symmetrical T wave inversion) .
3. Elevation of serum CK-MB levels more than the normal range .

EXCLUSION CRITERIA

1.Anaemia

2.Infection

3. Malignant disease

4 . Collagen disease

5. Stroke

6. Cardiac disease other than coronary disease

7. Ballon angioplasty

8. Overt right or left ventricular failure

9. Thrombolytic treatment within three months of study

BLOOD COLLECTION

Blood samples were collected by venepuncture with strict aseptic precaution as soon as the subjects got admitted as per the inclusion criteria. All the blood samples were centrifuged at 3000 rpm for 10 minutes and serum separated.

One part of the serum sample was taken for analysis of CK-MB , Creatinine, and Urea.

The remaining part of the serum sample was stored for analysis of soluble CD40L at -40ºC

12-14 hours fasting blood sample was also collected from all subjects during their hospital stay and analysis of total cholesterol, TGL, HDL-C, fasting blood sugar were done.

ANALYSIS OF BLOOD SAMPLES:

The serum sample collected above was used for the estimation of the following parameters.

A) ESTIMATED PARAMETERS

1. Serum sCD40L- Enzyme Linked Immuno Sorbant Assay
2 Serum.CK-MB – Kinetic immuno inhibition method
3. FBG- Trinders method, end point, fixed time
4. Blood Urea- Urease – GLDH method
5. Serum Creatinine - Jaffes method, initial rate
6. Serum TC - Cholesterol oxidase-PAP, end point
7. Serum TGL - GPO-PAP method, end point
8. Serum HDL-C - Phosphotungstic acid method, end point
9. Serum LDH - Modified IFCC Method
10. Serum AST- DGKC Method, Kinetic

B) CALCULATED PARAMETERS

S. LDL-C and S. VLDL-C are calculated using Friedewald's formula:

VLDL-C = TGL/5

LDL-C = TC- [HDL+(TGL/5)]

HUMAN SERUM SOLUBLE CD 40 LIGAND ASSAY:

PRINCIPLE OF THE ASSAY:

This assay is an in vitro enzyme- linked immunosorbant assay for the quantitative measurement of human CD40 in serum, plasma. This assay employs an antibody specific for human CD40 coated on a 96 –well plate.

Standards and samples are pipetted into the wells and CD40 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human CD40 antibody is added.

After washing away unbound biotinylated antibody , HRP-conjugated streptavidin is pipetted into the wells . The wells are again washed , a TMB substrate solution is added to the wells and color develops in proportion to the amount of CD40 bound. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm.

REAGENTS:

1. CD40 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with antihumanCD40
2. Wash Buffer Concentrate (20 x) (Item B): 25 ml of 20 x concentrated solution.
3. Standards (Item C): 2 vials , recombinant human CD40.

4. Assay Diluent D (Item K) : 15 ml of 5x concentrated buffer. For Standard /Sample (Serum/plasma samples /cell culture medium/urine) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For detection antibody and HRP –Streptavidin diluent.
6. Detection Antibody CD40 (Item F): 2 vial of biotinylated anti-human CD40 (each vial is enough to assay half micropalte)
7. HRP- Streptavidin concentrate (Item G): 8 µl 25,000x concentrated HRP-concentrated streptavidin.
8. TMB One –Step Substrate Reagent (Item H): 12 ml of 3,3,5,5 tetramethylbenzidine (TMB) in buffered solution.
9. Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

STORAGE:

1. The components of the kit are stored at 2-8º C.
2. Standard (recombinant protein) should be stored at -20º C or -80º C after reconstitution.
3. Opened Microplate Wells or reagents may be stored for upto 1 month at 2-8º C.

SAMPLE COLLECTION, SEPARATION, STORAGE:

Serum:

The blood samples were collected and centrifuged at 3000 rpm for 10 minutes.

Then serum samples were separated and stored at -20º C.

REAGENT PREPARATION:

1. Bring all reagents and samples to room temperature (18-25º C) before use.

2. Assay Diluent D (Item K) and Assay Diluent B (Item E) should be diluted 5-fold with deionized water or distilled water before use.

3. PREPARATION OF STANDARD:

Briefly spin the vial of Item C. Add 400 µl 1x Assay Diluent D (Item K) into Item C vial to prepare a100ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 180 µl CD40 standard from the vial of Item C, into a tube with270µl 1xAssay Diluent D to prepare a 40,000 pg/ml standard solution. Pipette 400µl 1xAssay Diluent D into each tube .Use the stock standard solution to produce a dilution series. Mix each tube thoroughly before the next transfer. Gently vortex to mix. 1xAssay Diluent D serves as the zero standard (0 pg/ml)

STANDARD POINT	DILUTION	SCD40 LIGAND (ng/ml)
S1	180µl standard+270µl 1xAssay Diluent D	40 .00
S2	200µl S1 +400 µL 1x Assay Diluent D	13.33
S3	200µl S2 +400µl 1x Assay Diluent D	4.44
S4	200µl S3 + 400 µl 1x Assay Diluent D	1.48
S5	200µl S4 +400µl 1xAssay Diluent D	0.493
S6	200µl S5 + 400µl 1x Assay Diluent D	0.164
S7	200µl S6 +400µl 1x Assay Diluent D	0.054
S8	400µl 1x Assay Diluent D	0.0

4. Wash concentrate (20x) (Item B) contains visible crystals , warm to room temperature and mix gently until dissolved .Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer (1:20).
5. Briefly spin the Detection Antibody vial (Item F) before use. Add 100µl of 1x Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently .The detection antibody should be diluted 80 fold with 1xAssay Diluent B .

6. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. Add 2µl of HRP-Streptavidin concentrate into a tube with 198µl 1x Assay Diluent B to prepare a 100 fold diluted HRP-Streptavidin .Mix through and then pipette 40µl of prepared 100 –fold diluted solution into a tube with10 ml 1x Assay Diluent B to prepare a final 25,000 fold diluted HRP Streptavidin solution.

ASSAY PROCEDURE:

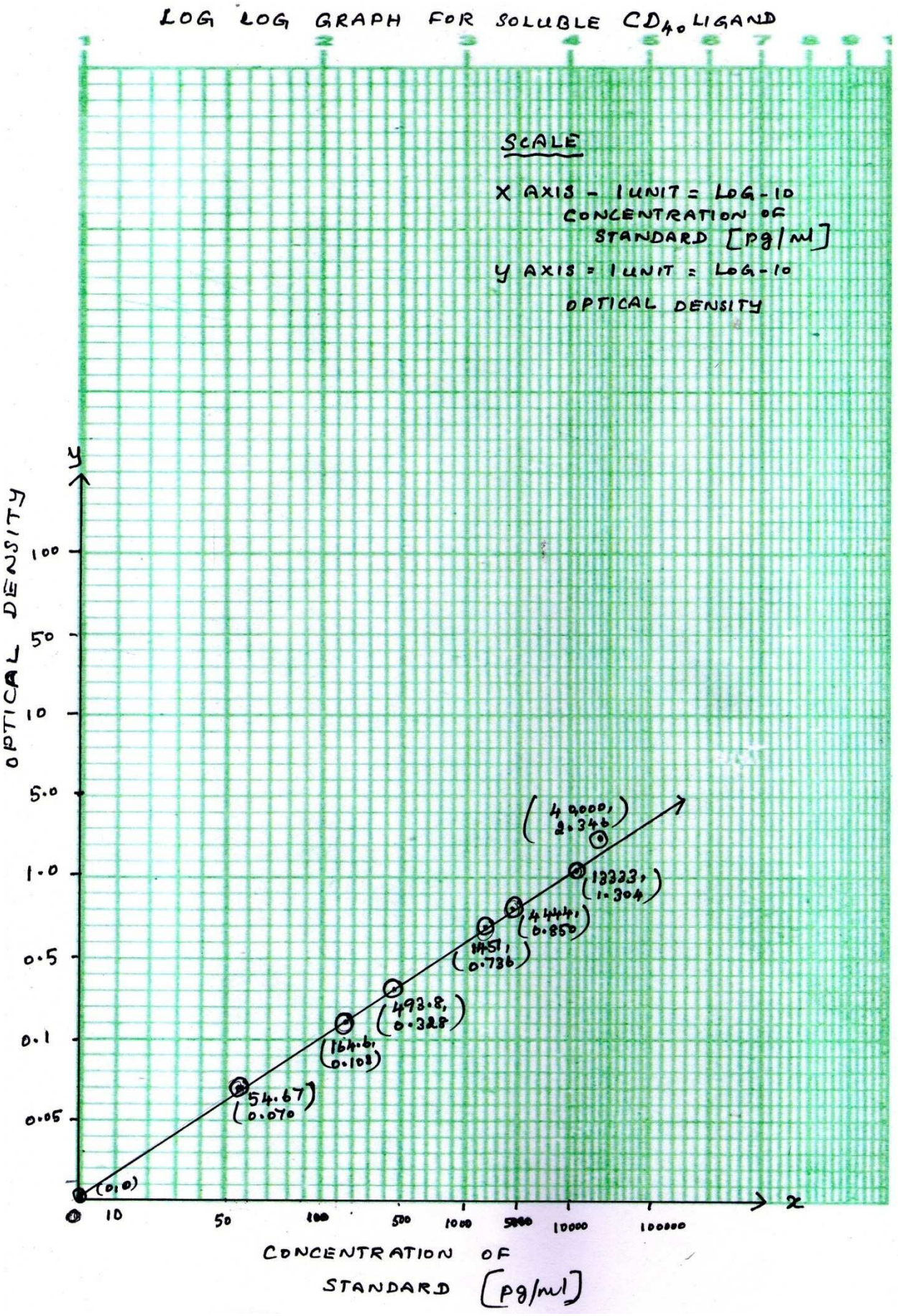
1. Bring all reagents and samples to room temperature (18-25º C) before use. It is recommended that all standards and samples be run in duplicate.
2. Add 100µl of each standard and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature .
3. Discard the solution and wash 4 times with 1x Wash Solution . Wash by filling each well with Wash Buffer (300µl) using a multi-channel Pipette. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100µl of 1x prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution and wash 4 times with 1xWash Solution (300µl) using a multi- channel Pipette .Invert the plate and blot it against clean paper towels.
6. Add 100µl of prepared Streptavidin solution to each well .Incubate for 45 minutes at room temperature.
7. Discard the solution and wash with 1x Wash solution (300µl) using a multi-channel Pipette .Invert the plate and blot it against clean paper towels.
8. Add 100µl of TMB One –Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

DATA ANALYSIS:

The mean absorbance for each set of duplicate standards, controls, and samples were calculated and the average zero standard optical density was subtracted. The standard curve on log-log graph paper was plotted, with standard concentration on the x-axis and absorbance on the y-axis.

Plasma reference value- 1.57±0.78 ng/ml.


LOG LOG GRAPH FOR SOLUBLE CD40 LIGAND
SCALE
X AXIS - 1 UNIT = LOG-10 CONCENTRATION OF STANDARD [pg/ml]
Y AXIS = 1 UNIT = LOG-10 OPTICAL DENSITY
OPTICAL DENSITY
y
100
50
10
5.0
1.0
0.5
0.1
0.05
(40000, 2.346)
(13333, 1.304)
(4444, 0.850)
(1481, 0.736)
(493.8, 0.328)
(164.6, 0.108)
(54.67, 0.070)
(0,0)
0
10
50
100
500
1000
5000
10000
100000
x
CONCENTRATION OF STANDARD [pg/ml]

ESTIMATION OF SERUM CREATINE KINASE-MB:

Methodology: Kinetic immuno inhibition method

Principle:

This procedure involves measurement of CK activity in the presence of antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB while not affecting B subunit activity of CK-MB and CK-BB. Then the CK method is used to quantitatively determine CK-B activity. The CK-MB activity is obtained by multiplying the CK-B activity by 2.

$$\text{Creatine phosphate + ADP} \xrightarrow{\text{Creatine Kinase}} \text{Creatine + ATP}$$

$$\text{ATP + D-glucose} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate + ADP}$$

$$\text{Glucose-6-Phosphate + NADP + } H^{+} \xrightarrow{\text{G-6-PDH}} \text{6-Phospho gluconate + NADPH}$$

G-6-PDH – Glucose -6-phosphate dehydrogenase

Sample: Unhemolysed serum used

Reagents:

Reagent 1 – Buffer/enzymes

Reagent 1A – Anti human polyclonal CKM antibody (goat)

Reagent Reconstitution – Reagents were allowed to attain the room temperature. 3ml of Reagent 1A was added to 1 bottle of Reagent1. Mixed gently till it was completely dissolved and waited for 5 min.

Procedure:

	Test
Reconstituted reagent	1ml
Sample	50µl

Mixed well and read immediately at wavelength of 340 nm.

Reference values – Serum - 0 to25 IU/L

ESTIMATION OF BLOOD GLUCOSE

Method: Trinders method, end point/fixed time

Principle:

Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase.

The enzyme peroxidase catalyses oxidative coupling of 4-amino antipyrine with phenol to yield a colored quinoneimine complex, with absorbance proportional to concentration of glucose in the sample.

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{Gluconoic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4AAP \longrightarrow \text{Quinoimine dye} + 2H_2O_2$$

Glucose standard: 100 mg/dl

Specimen : Fresh unhemolysed serum used.

Assay procedure:

Pipette into test tube labeled as	Blank	Standard	Test
Sample	-	-	10µl
Standard	-	10 µl	-
Enzyme reagent	1 ml	1 ml	1 ml
Distilled water	10 µl	-	-

Mixed well after each addition and incubated at 37º C for 5 minutes. The absorbance of the standard and the test were read against reagent blank at 505 nm.

CALCULATION:

Glucose (mg/dl) = absorbance of test/ absorbance of standard X concentration of standard (100 mg/dl)

Linearity upto 500 mg/dl by end point method.

NORMAL VALUES:

Glucose fasting 65-110 mg/dl

Glucose postprandial 90-130 mg/dl

ESTIMATION OF BLOOD UREA:

METHOD: Urease-GLDH method

PRINCIPLE:

Urea in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction catalyzed by glutamate dehydrogenase (GLDH) converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of reduced NADH to NAD. Two moles of NADH are oxidized for each mole of urea present.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-Oxoglutarate} \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+$$

The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample.

REAGENT COMPOSITION:

Reagent 1: α-ketoglutaric acid 99.8 mmol/L, Urease 23.5 KU/L, GLDH 3.5 KU/L, Adenosine diphosphate 7.6 mmol/L, Sodium azide 0.2%

Reagent 2: NADH 2.95 mmol/L, Sodium azide 0.1%

REAGENT PREPARATION:

Working reagent was prepared by mixing 4 parts of reagent 1 with one part of reagent 2.

PROCEDURE:

PIPETTE INTO TUBES	BLANK	STANDARD	TEST
Working Reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Test	-	-	10 µl

Mixed well and the absorbance was read after 30 seconds (A_1) and 60 sec (A_2) at 340 nm.

CALCULATION:

$\Delta A = A_2 - A_1$

$$\text{Urea (mg/dl)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \text{ x Concentration of standard (50 mg/dl)}$$

LINEARITY: The method is linear upto 200 mg/dl

REFERENCE INTERVAL: 15-30 mg/dl

ESTIMATION OF SERUM CREATININE:

Method: Jaffes method, initial rate

Methodology: Modified Jaffes reaction

PRINCIPLE:

Creatinine present in the sample reacts with picric acid in alkaline medium forming creatinine picrate(red colored complex) which is measured photometrically at 490 nm.

Reagent composition;

Reagent 1- picric acid reagent

Reagent 2- sodium hydroxide

Creatinine standard-2 mg/dl

Reagent preparation:

Equal volume of reagent 1 and 2 were mixed and allowed to wait for 15 min before use.

Sample: Unhemolysed serum use

Assay procedure:

Pipette into tubes marked	Standard	Test
Working reagent	1000µl	1000µl
Standard	100µl	-
Test	-	100µl

Mixed well and initial absorbance (A1) was read at 20 sec after mixing and final absorbance (A2) after 80 sec after mixing.

CALCULATION:

Delta A =A2-A1

Creatinine (mg/dl) = delta A of the test /delta of the standard x concentration of the standard (mg/dl)

Linearity – upto20 mg/dl

ESTIMATION OF SERUM TOTAL CHOLESTEROL

Method : Cholesterol oxidase-PAP, endpoint

Principle:

1. Cholesterol ester +Water $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

Cholesterol oxidase

2. Cholesterol + Oxygen $\longrightarrow$ Cholest-4-en-3one + H_2O_2

3. $2H_2O_2$ + 4AAP+ Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + $4H_2O$

- 4AAP- 4 amino antipyrine
- Absorbance of quinoneimine formed is directly proportional to cholesterol concentration.
- Cholesterol standard – 200mg/dl
- Sample: Unhemolysed serum

REAGENT COMPOSITION: ready for use

Goods buffer (PH-6.4) : 100mmol/L

Cholesterol oxidase : >100U/L

Cholesterol esterase : >200U/L

Peroxidase : >3000U/L

4-Aminoantipyrine : 0.3mmol/L

Phenol : 5mmol/L

ASSAY PROCEDURE:

REAGENTS	**BLANK**	**STANDARD**	**TEST**
Working reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl
Distilled water	10 µl	-	-

Mixed well and incubated for 10 min at room temperature. The absorbance of the test and standard were read against reagent blank at wavelength of 505 nm.

CALCULATION:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard (mg/dl)}$$

REFERENCE RANGE:

SERUM/PLASMA	**mg/dl**
2-12 months	60-190
≥ 1 year	110-230
Adults	< 200

Linearity-upto 700mg/dl

Sensitivity-1mg/dl

Interference:

Hb upto 200mg/dl, ascorbate upto 12mg/dl, bilirubin upto 10mg/dl and Triglycerides upto 700 mg/dl do not interfere with the test.

TRIGLYCERIDES:

ESTIMATION OF SERUM TRIGLYCERIDES:

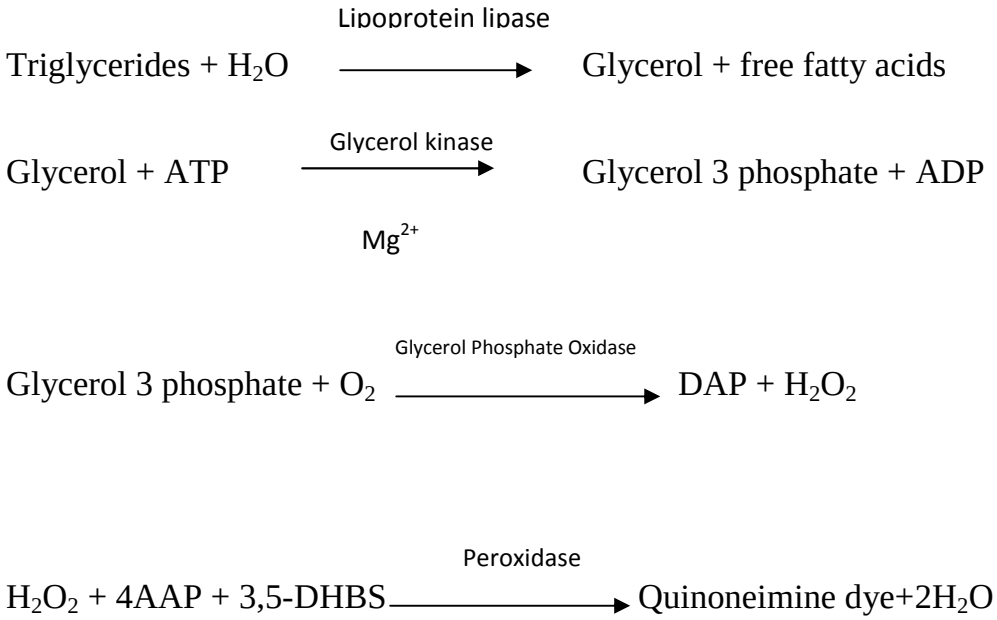
Method: GPO-PAP method, endpoint

Methodology:

Colorimetric, enzymatic method with glycerol phosphate oxidase.

This reagent is based on the method of Wako and the modifications by Mc Gowan et al.. and Fossati et al..

PRINCIPLE:



ATP- Adenosine Tri Phosphate

4AAP- 4Amino Anti Pyrine

DHBS-3,5 Dichloro-2Hydroxy Benzene Sulfonate

The intensity of Quinoneimine dye formed is proportional to the triglyceride concentration in the sample when measured at 505 nm (500-540nm).

Triglycerides standard concentration- 200mg/dl

REAGENT COMPOSITION

Reagent 1(Enzymes / Chromogen)

Lipoprotein lipase	4000 U/L
4-Amino antipyrine	0.4 mmol/L
ATP	2mmol/L
Glycerol kinase	1500 U/L
Peroxidase	2200 U/L
Glycerol Phosphate Oxidase	4000 U/L

REAGENT 2

Pipes buffer, PH-7.0 : 40mmol/L

DHBS:0.2mmol/L

Magnesium salt: 2.5mmol/L

Working reagent preparation:

The working reagent was prepared by mixing 4 parts of R1 with 1 part of R2, stable for 90 days at 2-8 ˚C.

Sample: Unhemolysed serum collected after 12 hrs of fasting.

ASSAY PROCEDURE:

Reagents	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl
Distilled water	10 µl	-	-

Mixed well and incubated for 10min.

Absorbance were read at 505nm for standard and sample against reagent blank.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard (mg/dl)}$$

Reference values:

Serum/plasma	37°C
Normal fasting level	25-160mg/dl

Linearity – upto 1000mg/dl

Sensitivity- 2mg/dl

Specificity/interference:

Hb upto 300mg/dl, ascorbate upto 3mg/dl, bilirubin upto 20mg/dl

ESTIMATION OF SERUM HDL CHOLESTEROL

Phosphotungstic acid method, endpoint

Principle:

Chylomicrons, LDL and VLDL are precipitated from serum/phospho tungstate in the presence of divalent cations such as Magnesium. The HDL cholesterol reminds unaffected in the supernatant and is estimated using cholesterol reagent.

$$\text{Serum/plasma} \xrightarrow[Mg^{2+}]{\text{Phosphotungstate}} \underset{\text{(supernatant)}}{\text{HDL}} + \underset{\text{(Precipitate)}}{(\text{LDL} + \text{VLDL} + \text{Chylomicrons})}$$

Reagent composition:

Reagent1: precipitating reagent

Phosphotungstic acid	2.4mmol/l
Magnesium chloride	40mmol/l

HDL cholesterol standard – 25mg/dl

SAMPLE: Unhemolysed serum used

PRECIPITATION:

Precipitation of LDL, VLDL and Chylomicrons done as follows:

Pipette	**Volume**
Test	250µl
Precipitating reagent	500 µl

Mixed well and the reaction mixture was allowed to stand for 10 min at room temperature, centrifuged at 4000 rpm for 10 min and obtain a clear supernatant. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

ASSAY PROCEDURE:

Reagents	**Blank**	**Standard**	**Test**
Cholesterol working reagent	1000µl	1000 µl	1000µl
Distilled water	50 µl	-	-
HDL standard	-	50 µl	-
Supernatant	-	-	50 µl

Mixed well and incubated for 10 min at room temperature.

The absorbance of the standard and the test samples were read at 505 nm against reagent blank.

Calculation:

$$\text{HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Concentration of standard (mg/dl) x dilution factor}$$

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x 25 x 3}$$

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 75$$

Linearity - upto 125mg/dl

Normal values:

Males - 30 to 65mg/dl

Females – 35 to 80mg/dl

Interference:

High triglyceride concentration above 300 mg/dl cause interference with the assay.

Bilirubin and ascorbate at high concentrations interfere with precipitation

FRIEDEWALD'S EQUATION FOR CALCULATION OF SERUM LDL CHOLESTEROL

TGL/5 = VLDL, if TGL is less than 400mg/dl

LDL-C = Total cholesterol – (HDL -C+ VLDL-C)

ESTIMATION OF SERUM LACTATE DEHYDROGENASE (LAH-P)

DGKC, METHOD, KINETIC

METHODOLOGY

Method of Henry et.al[2.]

PRINCIPLE

When pyruvate is converted to Lactate in the presence of NADH , oxidation of NADH to NAD is measured at 340 nm and the decrease in absorbance is directly propotional to LDH activity

REAGENT COMPOSITION (When reconstituted as directed)

REAGENT 1: LDH Reagent

Pyruvic acid	0.60 mmol/L
NADH(Yeast)	0.23 mmol/L
Phosphate buffer	pH7.5

Also contains non-reactive filters and stabilizers.

REAGENT RECONSTITUTION

The reagent and the Aqua-4 (supplied in the kit) is allowed to attain room temperature, the amount of Aqua-4 indicated on the label is added to contents of each vial, swirled to dissolve and should not be shaken vigorously.

ASSAY PROCEDURE

Pipette	Volumes
Working Reagent	1000µl
Test	20 µl

Mixed well and aspirated.

CALCULATION

The absorbance change/min (Δ A/Min.) for every reading is calculated and the mean value is found out.

$$\text{IU/L} = \frac{\left(\frac{\Delta A}{Min}\right) \times T.V \times 10^3}{S.V \times Absorptivity \times P}$$

Where:

T.V. = Total reaction Volume in µl

S.V. = Sample Volume in µl

Absorptivity = millimolar absorptivity of NADH at 340 nm

= 6.22

P = cuvette light path (cm)

=1cm

Activity of LDH-P at 37^0 C (IU/L) = (A_{340} /min)x Factor(8199)

LINEARITY:

Upto 1000IU/L . For higher values it is recommended to dilute the samples with normal saline and repeat the assay . Multiply the final results with the dilution factor.

NORMAL VALUES:

Serum LDH level = 180-360 U/L .

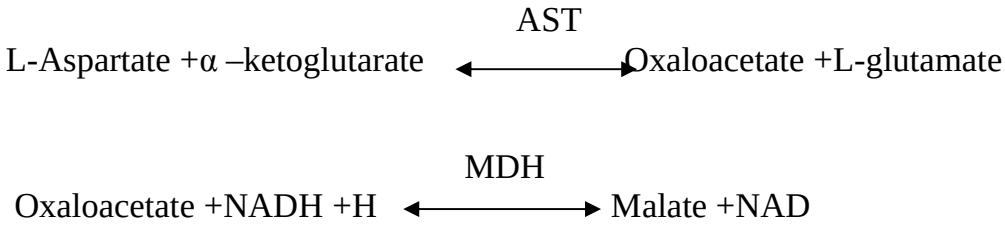
ESTIMATION OF SERUM ASPARTATE AMINOTRANSFERASE

Methodology: Modified IFCC Method

Principle:

The transfer of amino group from L- Aspartate to α- ketoglutarate to yield oxaloacetate and L – Glutamate is catalyzed by AST. When Oxaloacetate undergoes reduction there is simultaneous oxidation of NADH to NAD in the presence of MDH.

The decrease in the rate of absorbance at 340nm is directly proportional to the AST activity. Interference from endogenous pyruvate which is normally present in serum is prevented by the addition of LDH.



REAGENT COMPOSITION

R1:

Tris Buffer (pH 7.8) – 20 mmol/L

L-Aspartate - 230mmol/L

LDH - > 33.3µkat/L

2- Oxoglutarate - 13.21 mmol/L

MDH - 3.333µkat/L

Also contains Non –reactive fillers and stabilizers.

R2:

NADH - 1.51MMOL/L

REAGENT PREPARATION:

Prepare the working reagent by mixing 4 parts of R1 with 1part of R2 per assay tube.

ASSAY PROCEDURE:

Pipette	Volumes
Working reagent	500µl
Test	25 µl

CALCULATION:

AST ACTIVITY (IU/L) = D A/min. x Factor(3376)

LIMITATIONS:

1.Sample with values above 1600 IU/L should be diluted 1:1 with saline, reassayed and the results multiplied by two.

2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST , presumably due to a lack of pyridoxal phosphate.

LINEARITY:

AST reagent is linear upto 1600 U/L . For values above the linearity limit dilute with saline and reassay. Multiply by the dilution factor to obtain the end result.

EXPECTED VALUES:

Adult Male: <35 U/L

Adult Female: <31 U/L

STATISTICAL ANALYSIS

- Student T –test and Chi- square test were employed for the statistical analysis of data.
- The data were expressed in terms of mean and standard deviation.
- 'P' value less than 0.05 was taken as significant value.
- Correlation between the measured parameters was assessed using Pearson's coefficient of correlation.

MASTER CHART 1 - CONTROL GROUP

S.NO	AGE	SEX	WEIGHT (kg)	HEIGHT (m)	BMI	SBP mm Hg	DBP mm Hg	SCD 40L ng/ml	CKMB U/L	FBG mg/dl	UREA mg/dl	CREAT mg/dl	TC mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	LDH U/L	AST U/L
1	65	F	64	1.6	25	122	82	1.26	7	96	29	0.5	193	100	43	130	20	120	12
2	48	F	50	1.5	22	110	70	1.24	14	93	28	0.9	188	115	25	140	23	140	14
3	62	F	70	1.65	26	100	80	1.34	12	91	32	0.8	179	160	31	116	32	136	19
4	67	F	60	1.53	26	120	80	1.39	10	90	18	0.8	159	130	52	81	26	145	20
5	44	F	65	1.65	24	110	80	1.44	7	98	22	0.7	158	133	39	92	27	155	14
6	57	F	55	1.48	25	100	82	1.41	16	92	36	0.6	183	138	51	114	28	180	15
7	61	F	60	1.48	27	122	70	1.36	17	98	20	0.7	165	145	49	87	29	135	18
8	54	F	63	1.45	30	123	72	1.52	20	96	24	0.8	195	135	50	118	27	125	12
9	63	F	60	1.55	25	130	76	1.5	18	89	28	0.5	145	120	43	78	24	140	11
10	55	F	62	1.54	26	100	80	1.54	20	90	30	0.6	172	113	41	108	23	128	13
11	46	F	55	1.5	24	120	80	1.28	16	92	20	0.7	171	122	40	107	24	149	14
12	37	F	58	1.52	25	114	74	1.32	12	94	28	0.9	186	126	49	112	25	138	19
13	50	F	65	1.55	27	110	70	1.4	10	98	32	0.8	156	130	32	98	26	126	18
14	65	F	55	1.6	21	130	80	1.51	14	86	18	0.5	173	145	45	99	29	142	16
15	45	F	60	1.52	26	110	80	1.56	12	92	22	0.6	171	122	40	107	24	138	12
16	60	F	60	1.53	26	126	84	1.25	13	96	24	0.7	141	115	43	75	23	130	17
17	52	F	58	1.52	25	124	80	1.37	17	90	22	0.5	176	170	40	102	34	145	13
18	40	F	55	1.48	25	110	80	1.64	15	96	32	0.8	179	160	31	116	32	138	14
19	55	F	55	1.6	21	128	80	1.34	20	88	30	0.6	127	150	25	72	30	128	15
20	45	F	72	1.67	26	122	80	1.26	22	98	20	0.7	158	133	39	92	27	152	13
21	45	M	65	1.65	24	126	82	1.57	16	86	22	0.8	178	141	24	126	28	142	18
22	47	M	60	1.67	22	120	80	1.55	11	84	30	0.7	138	116	40	75	23	136	12
23	57	M	70	1.7	24	112	74	1.35	16	90	28	0.7	176	115	52	101	23	148	11

24	68	M	75	1.6	29	130	80	1.34	17	80	26	0.6	188	98	53	115	20	127	18
25	39	M	70	1.6	27	120	80	1.27	12	98	24	0.8	190	111	33	135	22	136	19
26	44	M	70	1.68	25	128	74	1.37	14	90	26	0.8	190	111	35	133	22	139	10
27	62	M	80	1.7	28	110	70	1.39	14	102	22	0.7	173	145	45	99	29	140	17
28	58	M	69	1.65	25	120	80	1.61	16	104	20	0.6	175	100	42	113	20	137	13
29	48	M	75	1.68	27	116	84	1.3	10	98	30	0.5	154	155	49	74	31	143	18
30	38	M	75	1.72	25	110	72	1.4	8	84	34	0.9	167	141	47	92	28	123	12
31	47	M	72	1.69	25	110	70	1.66	16	96	24	0.7	150	86	35	97	18	131	19
32	53	M	70	1.65	26	122	82	1.58	14	102	28	0.7	152	126	60	67	25	122	11
33	64	M	69	1.6	27	110	80	1.49	12	92	22	0.8	160	140	66	66	28	136	15
34	44	M	70	1.67	25	126	80	1.27	10	82	32	0.5	186	138	52	106	28	149	12
35	40	M	75	1.71	26	120	82	1.59	14	106	26	0.7	145	72	59	72	14	138	18
36	66	M	64	1.6	25	126	84	1.67	16	106	30	0.6	150	86	45	87	18	134	14
37	37	M	74	1.68	26	110	80	1.22	11	104	24	0.9	173	97	24	130	19	152	19
38	42	M	70	1.65	26	114	78	1.57	17	102	20	0.8	194	114	42	130	23	129	13
39	55	M	75	1.68	27	130	80	1.48	22	86	30	0.5	159	65	38	108	13	125	18
40	65	M	74	1.69	26	110	70	1.63	16	90	24	0.7	171	122	40	107	24	149	16
41	41	M	67	1.67	24	116	84	1.38	8	104	22	0.7	142	132	32	74	26	152	10
42	51	M	70	1.68	25	120	80	1.23	10	84	30	0.8	180	127	30	125	25	162	12
43	43	M	67	1.67	24	110	70	1.3	5	98	32	0.6	200	128	58	120	22	139	18
44	62	M	71	1.7	25	120	80	1.63	8	102	28	0.6	171	165	45	93	33	147	17
45	59	M	72	1.67	26	120	80	1.4	6	82	24	0.8	148	126	51	72	25	130	13
46	69	M	80	1.7	28	126	82	1.3	15	102	20	0.6	145	72	59	72	14	129	17
47	43	M	65	1.72	22	120	80	1.33	12	92	22	0.6	138	140	52	58	28	157	15
48	52	M	72	1.69	25	118	76	1.69	10	104	26	0.5	172	126	49	98	25	135	11
49	55	M	68	1.62	26	120	80	1.49	14	98	30	0.7	161	155	46	84	31	141	19
50	46	M	74	1.68	26	110	70	1.25	12	86	20	0.6	148	126	51	72	25	132	17

MASTER CHART 2 - STUDY GROUP

S.NO	AGE	SEX	Wt (Kg)	Ht (m)	BMI	SBP (mmHg)	DBP (mmHg)	DURATION (Hours)	SCD40L (ng/ml)	CK-MB (U/L)	FBG (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	TC (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	LDH (U/L)	AST (U/L)
1	57	F	55	1.5	24.44	100	70	6	3.904	48	86	24	0.8	242	150	31	181	30	134	10
2	55	F	64	1.56	26.29	150	100	4	3.812	46	98	20	0.7	240	152	33	177	30	191	22
3	60	F	60	1.55	24.97	110	80	6	4.739	52	108	32	0.6	280	186	32	211	37	142	12
4	55	F	65	1.52	28.13	100	80	4	3.944	48	118	28	0.7	243	168	34	176	33	149	15
5	58	F	56	1.55	23.3	140	100	6	7.259	60	98	26	0.8	278	240	38	200	40	192	24
6	55	F	60	1.53	25.63	110	70	4	3.386	46	220	18	0.7	287	198	36	217	34	136	18
7	39	F	75	1.6	29.29	120	80	3	3.234	42	104	22	0.8	288	180	34	218	36	121	13
8	46	F	73	1.65	26.81	100	80	6	4.394	54	98	34	0.8	285	172	32	219	34	140	16
9	65	F	74	1.72	25.01	110	70	4	1.581	24	210	26	0.6	198	122	40	134	24	120	13
10	55	F	64	1.68	22.67	160	100	3	1.376	21	96	30	0.7	197	216	41	113	43	191	26
11	56	F	70	1.64	26.02	170	110	3	1.641	22	90	28	0.8	196	200	40	116	40	188	23
12	75	F	72	1.67	25.81	90	70	2	1.491	20	96	22	0.8	195	114	43	130	22	112	11
13	65	F	60	1.5	26.66	100	80	6	7.844	61	114	26	0.8	360	256	32	277	51	128	15
14	70	F	72	1.5	32	110	70	6	1.396	23	92	36	0.6	197	106	41	135	21	139	16
15	42	F	64	1.68	22.67	100	80	4	3.256	43	108	28	0.7	285	162	33	220	32	128	19
16	57	F	55	1.6	21.48	140	90	4	3.434	41	98	22	0.6	288	167	38	217	33	177	22
17	45	F	80	1.65	29.48	100	80	4	4.364	52	84	20	0.7	295	178	32	228	35	142	13
18	68	F	60	1.53	25.63	110	80	6	5.709	54	176	18	0.6	285	189	32	215	38	139	12
19	56	F	65	1.48	29.67	170	110	6	7.934	62	94	24	0.7	234	243	33	153	48	176	25
20	60	F	56	1.55	23.3	120	80	5	8.099	64	112	20	0.8	249	276	34	160	55	122	18
21	42	M	55	1.72	18.59	140	90	6	4.969	46	92	22	0.7	288	202	30	208	50	172	24
22	55	M	70	1.64	26.02	110	80	4	3.104	40	104	30	0.6	289	156	33	225	31	154	19
23	50	M	83	1.67	29.76	110	70	6	3.554	42	210	26	0.7	290	165	32	225	33	137	13
24	48	M	75	1.6	29.29	100	70	3	3.506	45	108	18	0.8	298	176	34	229	35	129	11
25	63	M	68	1.62	25.91	152	94	3	1.506	22	98	20	0.7	192	132	42	124	26	164	22
26	45	M	86	1.64	31.97	180	110	4	5.939	68	88	24	0.8	287	276	30	202	55	190	26
27	66	M	62	1.64	23.05	120	80	6	6.344	96	92	28	0.7	289	266	31	205	53	127	16
28	65	M	64	1.6	25	110	70	4	7.634	76	86	32	0.6	310	265	34	223	53	134	18
29	65	M	55	1.52	23.8	100	80	5	5.055	67	94	24	0.8	288	187	33	218	37	151	14
30	54	M	62	1.68	21.96	110	90	6	5.594	66	78	26	0.7	285	183	32	217	36	122	12
31	50	M	61	155	25.39	120	80	3	1.661	14	96	34	0.7	188	125	41	122	25	147	19
32	45	M	76	1.72	25.68	144	96	4	4.424	67	102	28	0.8	285	210	30	213	42	191	24
33	53	M	73	1.67	26.17	110	80	6	4.436	68	188	18	0.6	260	232	31	183	46	140	15
34	50	M	65	1.67	23.3	120	80	3	5.369	66	90	20	0.8	275	190	31	206	38	139	19
35	67	M	64	1.65	23.5	110	70	6	8.009	84	120	22	0.8	345	199	30	275	40	141	13
36	56	M	62	1.7	21.45	100	80	5	6.449	98	118	28	0.6	289	186	35	217	37	153	14
37	46	M	65	1.6	25.39	110	80	3	1.536	18	92	32	0.6	190	145	40	121	29	135	15
38	50	M	67	1.65	24.6	90	70	4	4.484	70	104	24	0.6	278	184	36	205	37	122	18
39	67	M	62	1.7	21.45	120	80	6	4.184	65	84	26	0.7	245	178	32	178	35	149	13
40	45	M	64	1.64	23.79	100	70	4	5.414	84	78	32	0.6	276	198	33	203	40	153	15
41	35	M	76	1.72	25.68	110	70	4	3.134	80	192	28	0.7	269	154	30	208	31	142	10
42	47	M	65	1.7	22.49	150	90	3	1.396	18	94	20	0.7	192	240	44	100	48	192	23
43	70	M	75	1.68	26.57	110	70	5	5.144	80	108	22	0.5	286	145	30	227	29	138	13
44	55	M	70	1.64	26.02	150	100	4	4.319	68	118	24	0.7	248	232	33	169	46	182	26
45	40	M	55	1.72	18.59	120	80	6	4.491	70	116	30	0.6	256	101	31	205	20	146	16
46	65	M	65	1.6	25.39	110	80	3	6.599	105	96	24	0.8	259	156	32	194	33	139	14
47	68	M	75	1.69	26.57	90	70	4	4.551	72	88	26	0.6	251	189	31	182	38	152	18
48	63	M	77	1.7	26.64	110	70	5	4.799	75	76	22	0.8	259	190	33	188	38	145	13
49	48	M	75	1.72	25.35	110	80	4	4.544	73	92	30	0.7	257	136	32	198	27	150	17
50	66	M	70	1.64	26.02	110	90	6	4.934	78	104	24	0.6	260	179	33	191	36	140	19

TABLE 1: DESCRIPTIVE STATISTICS OF CONTROL AND STUDY

SL.NO	VARIABLES	Control(n=50)				Study(n=50)			
		Min.	Max.	Mean	S.D	Min.	Max.	Mean	S.D
1	AGE(Yrs)	37	69	52.22	9.412	35	75	55.56	9.52
2	Wt(Kg)	50	80	66.60	7.191	55	86	66.84	7.75
3	Ht (mt)	1.45	1.72	1.61	0.07	1.48	1.72	1.6270	0.07
4	BMI	21.48	29.96	25.34	1.72	18.59	32.00	25.29	2.83
5	SBP (mm Hg)	100	130	117.62	8.002	90	180	118.72	22.18
6	DBP (mmHg)	70	84	78.08	4.462	70	110	82.00	11.56
7	S.sCD40Lng/ml	1.216	1.691	1.42	.139	1.37	8.09	4.39	1.90
8	S.CK-MB U/L	5	22	13.48	4.04	14	105	56.08	22.58
9	FBG mg/dl	80	106	93.94	6.97	76	220	110.12	35.34
10	B.UREA mg/dl	18	36	25.82	4.60	18	36	25.36	4.65
11	S.CREA mg/dl	.5	.9	.684	0.12	.5	.8	.698	.084
12	S.TC mg/dl	127	200	166.98	17.75	188	360	262.12	39.72
13	S.TGL mg/dl	65	170	124.76	23.67	101	276	185.04	43.41
14	S.HDL-C mg/dl	24	66	43.24	9.90	30	44	34.16	3.88
15	S.LDL-C mg/dl	58	140	98.90	21.36	100	277	191.16	40.13
16	S.VLDL-C mg/dl	13	34	24.86	4.75	20	55	36.80	8.75
17	S.LDH U/L	120	180	139.00	11.32	112	192	148.86	22.37
18	S.AST U/L	10	20	15.02	2.93	10	26	17.04	4.62

TABLE 2: AGE AND SEX DISTRIBUTION IN CONTROL AND STUDY GROUPS

SL.NO	Age	Control		Statistical inference	Study		Statistical inference
		Male (n=30)	Female (n=20)		Male (n=30)	Female (n=20)	
1	Below 40yrs	4	2	X^2=.521 Df=3 .914>0.05 Not Significant	2	1	X^2=8.576 Df=3 .035<0.05 Significant
2	41 to 50yrs	11	6		12	3	
3	51 to 60yrs	8	6		5	11	
4	61yrs & above	7	6		11	5	

BAR DIAGRAM - I

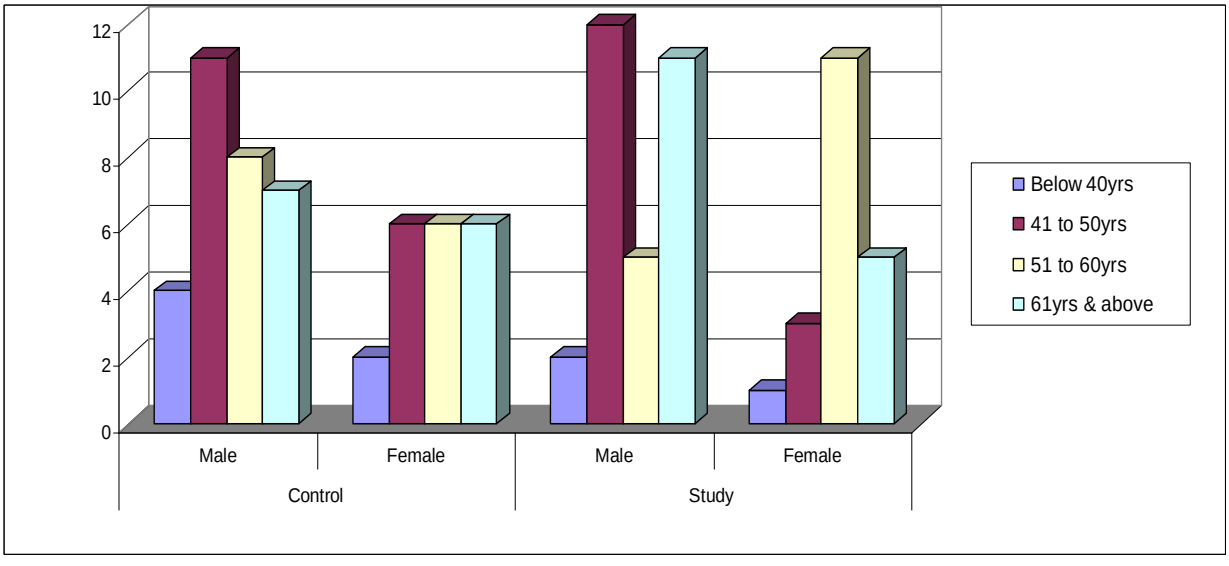


TABLE 3: MEAN S. sCD40L LEVELS IN CONTROL AND STUDY GROUPS

S.sCD40L ng/ml	MEAN	S.D	STATISTICAL INFERENCE
Control (n=50)	1.42250	0.139578	T=-11.024 P<0.001 Significant
Study (n=50)	4.39758	1.903162	

BAR DIAGRAM - II

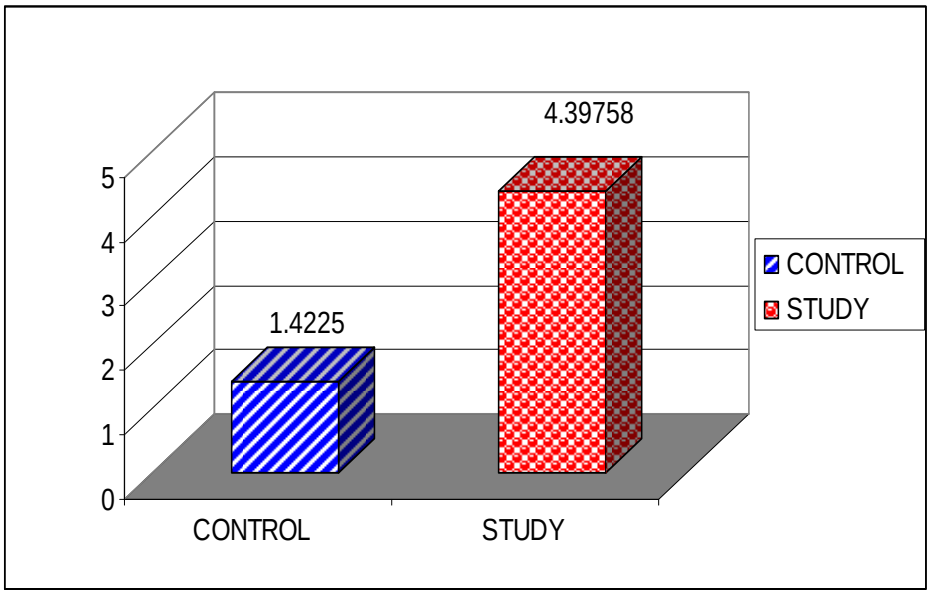


TABLE 4:

STATISTICAL ANALYSIS OF LIPID PARAMETERS BETWEEN CONTROL AND STUDY GROUPS

SLNO	VARIABLES	MEAN	S.D	STATISTICAL INFERENCE
1.	**S.TC mg/dl**			
	Control (n=50)	166.98	17.757	T=-15.461
	Study (n=50)	262.12	39.723	P < 0.001 Significant
2.	**S.TGL mg/dl**			
	Control (n=50)	124.76	23.671	T=-8.620
	Study (n=50)	185.04	43.413	P < 0.001 Significant
3.	**S.HDL-C mg/dl**			
	Control (n=50)	43.24	9.905	T=6.035
	Study (n=50)	34.16	3.883	P < 0.001 Significant
4.	**S.LDL-C mg/dl**			
	Control (n=50)	98.90	21.367	T=-14.349
	Study (n=50)	191.16	40.132	P < 0.001 Significant
5.	**S.VLDL-C mg/dl**			
	Control (n=50)	24.86	4.751	T=-8.479
	Study (n=50)	36.80	8.751	P < 0.001 Significant

BAR DIAGRAM III

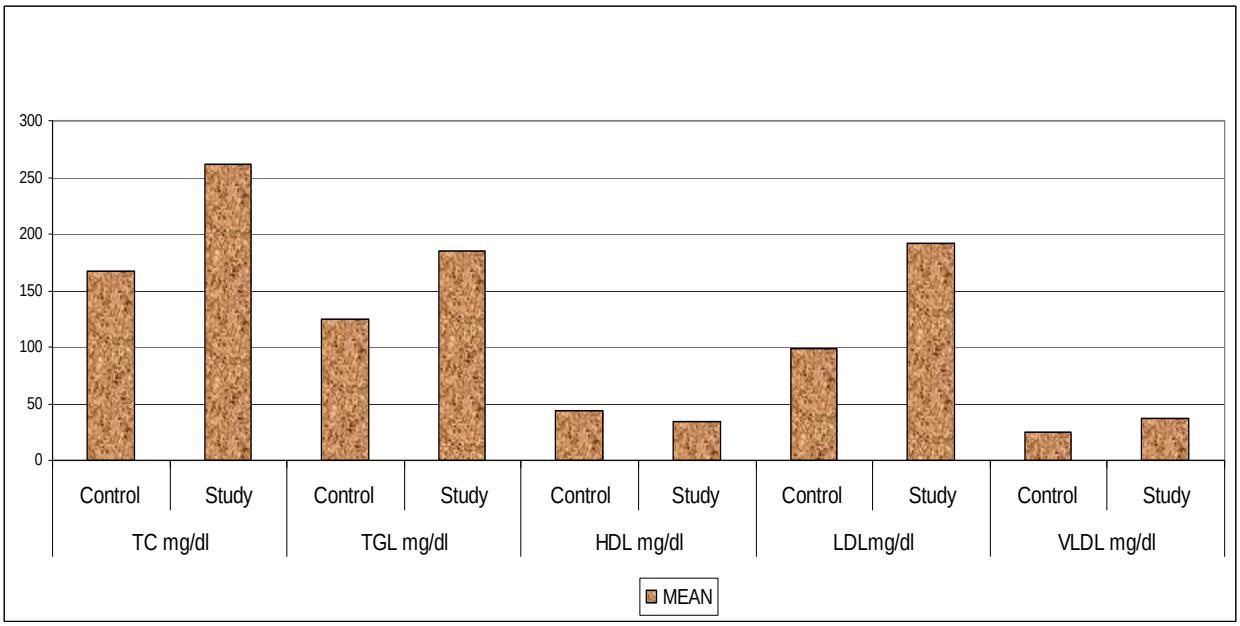


TABLE 5: MEAN S. CK-MB VALUES IN CONTROL AND STUDY GROUPS

SL.NO	S.CK-MB U/L	MEAN	S.D	STATISTICAL INFERENCE
1..	Control(n=50)	13.48	4.047	T=-13.129 P<0.05 Significant
2.	Study (n=50)	56.08	22.584	

BAR DIAGRAM-IV

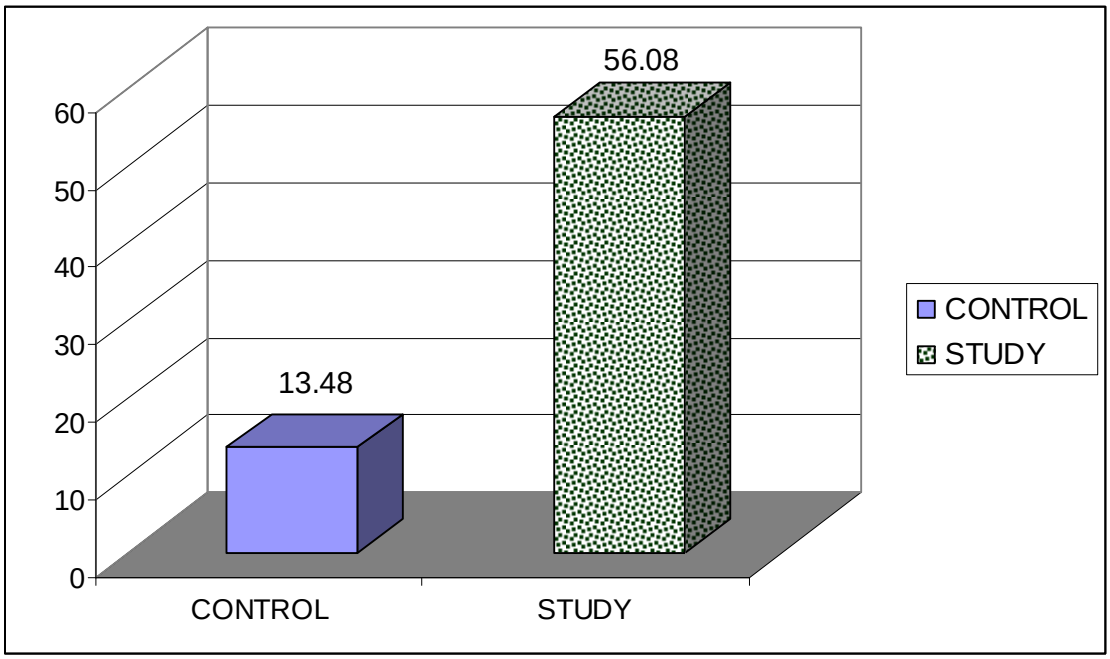
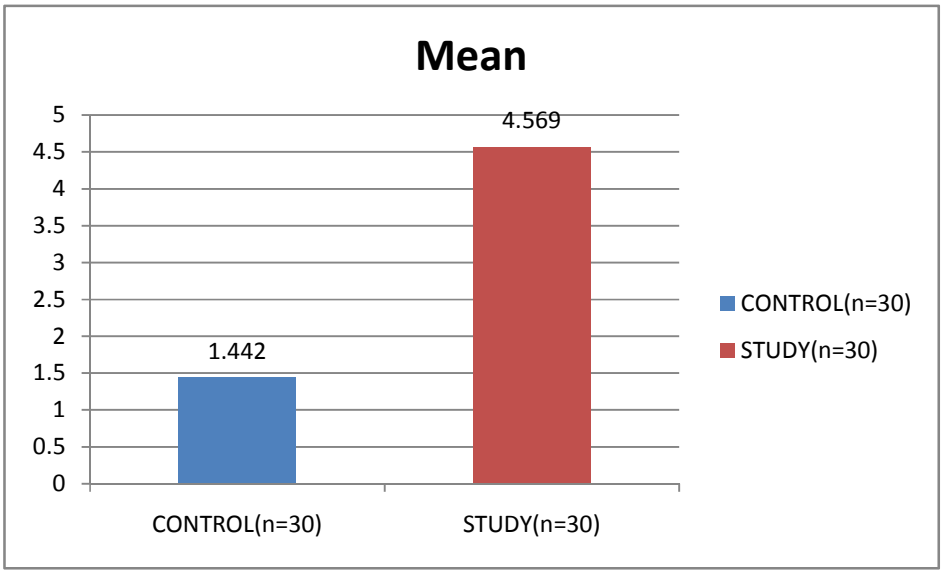


TABLE 6: MEAN LEVEL OF S. sCD40L LEVEL IN MALE CONTROL AND STUDY GROUP

S.sCD40L ng/ml	Mean	S.D	Statistical inference
Control(n=30)	1.442	0.151	T=-10.209
Study(n=30)	4.569	1.670	P<0.05Significant

BAR DIAGRAM - V



TBLE 7: MEAN S. sCD40L LEVEL IN FEMALE CONTROL AND STUDY GROUP

S.sCD40L ng/ml	Mean	S.D	Statistical inference
Control(n=20)	1.392	0.117	T=-5.507
Study(n=20)	4.139	2.227	P<0.05 Significant

BAR DIAGRAM-VI

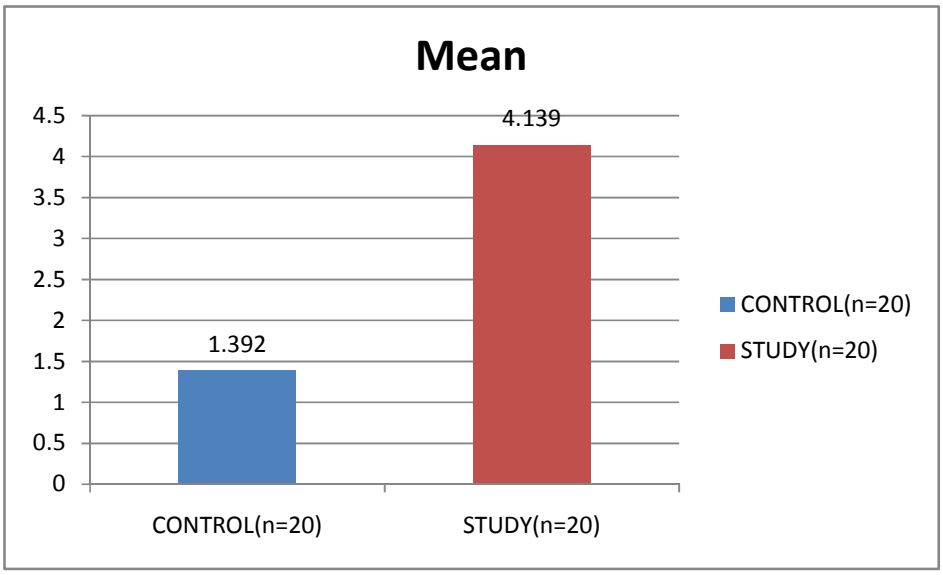


TABLE 8 –STATISTICAL ANALYSIS OF S. sCD40L, S.CK-MB AND LIPID PARAMETERS IN MALE CONTROL AND STUDY GROUP

SL.NO	VARIABLES	MEAN	S.D	STATISTICAL INFERENCE
1.	**S.sCD40L ng/ml**			
	Control (n=30)	1.44233	.151316	T=-10.209 P < 0.001 Significant
	Study (n=30)	4.56940	1.670877	
2.	**S.CK-MB U/L**			
	Control (n=30)	12.73	3.759	T=-11.752 P < 0.01 Significant
	Study (n=30)	64.03	23.611	
3.	**S.TC mg/dl**			
	Control (n=30)	165.80	17.924	T=-13.550 P < 0.001 Significant
	Study (n=30)	266.13	36.380	
4.	**S.TGL mg/dl**			
	Control (n=30)	119.20	25.656	T=-7.364 P < 0..001 Significant
	Study (n=30)	185.90	42.463	
5.	**S.HDL-C mg/dl**			
	Control (n=30)	45.13	10.641	T=5.746 P < 0.001 Significant
	Study (n=30)	33.30	3.743	
6.	**S.LDL-C mg/dl**			
	Control (n=30)	96.70	23.272	T=-12.322 P<0.001 Significant
	Study (n=30)	195.37	37.175	
7.	**S.VLDL-C mg/dl**			
	Control (n=30)	23.67	5.101	T=-7.459 P<0.001 Significant
	Study (n=30)	37.47	8.756	

TABLE 9 –STATISTICAL ANALYSIS OF S. sCD40L, S.CK-MB AND LIPID PARAMETERS IN FEMALE CONTROL AND STUDY GROUP

SL.NO	VARIABLES	MEAN	S.D	STATISTICAL INFERENCE
1.	**S.sCD40L ng/ml**			
	Control (n=20)	1.39275	.117230	T=-5.507 P < 0.001 Significant
	Study (n=20)	4.13985	2.227838	
2.	**S.CK-MB U/L**			
	Control (n=20)	14.60	4.297	T=-8.653 P < 0.01 Significant
	Study (n=20)	44.15	14.655	
3.	**S.TC mg/dl**			
	Control (n=20)	168.75	17.814	T=-8.140 P < 0.001 Significant
	Study (n=20)	256.10	44.562	
4.	**S.TGL mg/dl**			
	Control (n=20)	133.10	17.841	T=-4.601 P < 0.001 Significant
	Study (n=20)	183.75	45.884	
5.	**S.HDL-C mg/dl**			
	Control (n=20)	40.40	8.127	T=2.465 P<0.05 Significant
	Study (n=20)	35.45	3.818	
6.	**S.LDL-C mg/dl**			
	Control (n=20)	102.20	18.211	T=-7.697 P<0.001 Significant
	Study (n=20)	184.85	44.434	
7.	**S.VLDL-C mg/dl**			
	Control (n=20)	26.65	3.588	T=-4.276 P<0.001 Significant
	Study (n=20)	35.80	8.871	

TABLE 10: STATISTICAL ANALYSIS OF S. sCD40L, S.CK-MB, LIPID PARAMETERSAND S.LDH, S.AST IN CONTROL AND STUDY GROUP LESSTHAN 40YEARS

	VARIABLES	MEAN	S.D	STATISTICAL INFERENCE
1.	**S.sCD40L ng/ml**			
	Control (n=6)	1.40350	.174062	T=-7.286 P<0.001 Significant
	Study (n=3)	3.61967	.756252	
2.	**S.CK-MB U/L**			
	Control (n=6)	12.00	2.449	T=-6.853 P < 0.01 Significant
	Study (n=3)	64.00	19.698	
3.	**S.TC mg/dl**			
	Control (n=6)	173.33	16.207	T=-8.539 P<0.001 Significant
	Study (n=3)	271.00	16.093	
4.	**S.TGL mg/dl**			
	Control (n=6)	117.83	31.505	T=-1.122 P>0.05 Not Significant
	Study (n=3)	145.00	40.262	
5.	**S.HDL-C mg/dl**			
	Control (n=6)	40.50	13.233	T=1.112 P>0.05 Not Significant
	Study (n=3)	31.67	2.082	
6.	**S.LDL –C mg/dl**			
	Control (n=6)	109.50	23.797	T=-6.977 P < 0.001 Significant
	Study (n=3)	210.33	6.807	
7.	**S.VLDL-C mg/dl**			
	Control (n=6)	23.33	6.439	T=-1.148 P>0.05 Not Significant
	Study (n=3)	29.00	8.185	
8.	**S.LDH U/L**			
	Control (n=6)	137.50	9.203	T=.156 P>0.05 Not Significant
	Study (n=3)	136.33	13.429	
9.	**S.AST U/L**			
	Control (n=6)	16.83	3.061	T=1.781 P>0.05 Not Significant
	Study (n=3)	13.00	3.000	

TABLE 11: STATISTICAL ANALYSIS OF S. sCD40L, S.CK-MB, LIPID PARAMETERS AND S.LDH, S.AST IN 41-50 YEARS OF AGE IN CONTROL AND STUDY GROUP

SL.NO	VARIABLES	MEAN	S.D	STATISTICAL INFERENCE
1.	**S.sCD40L mg/dl**			
	Control (n=17)	1.39306	.138689	T=-7.244 P < 0.001 Significant
	Study (n=15)	3.92067	1.434151	
2.	**S.CK-MB U/L**			
	Control (n=17)	12.47	4.185	T=-7.191 P < 0.01 Significant
	Study (n=15)	50.67	21.490	
3.	**S.TC mg/dl**			
	Control (n=17)	165.88	20.448	T=-8.986 P<0.001 Significant
	Study (n=15)	264.60	39.787	
4.	**S.TGL mg/dl**			
	Control (n=17)	126.00	15.374	T=-5.664 P<0.001 Significant
	Study (n=15)	183.93	38.939	
5.	**S.HDL-C mg/dl**			
	Control (n=17)	40.29	9.610	T=2.332 P<0.05 Significant
	Study (n=15)	34.00	4.342	
6.	**S.LDL-C mg/dl**			
	Control (n=17)	100.06	24.322	T=-7.767 P<0.001 Significant
	Study (n=15)	193.27	42.230	
7.	**S.VLDL –C mg/dl**			
	Control (n=17)	25.00	3.102	T=-5.581 P<0.001 Significant
	Study (n=15)	37.33	8.508	
8.	**S.LDH U/L**			
	Control (n=17)	141.71	9.393	T=-1.509 P>0.05 Not Significant
	Study (n=15)	151.13	23.778	
9.	**S.AST U/L**			
	Control (n=17)	14.53	2.961	T=-2.700 P>0.05 Not Significant
	Study (n=15)	18.13	4.518	

TABLE 12: STATISTICAL ANALYSIS OF S. sCD40L, S.CK-MB,LIPID PARAMETERS AND S.LDH,S.AST IN IN CONTROLAND STUDY GROUP FOR THE AGE GROUP OF 51 TO 60 YEARS

SL.NO	VARIABLES	MEAN	S.D	STATISTICAL INFERENCE
1.	**S.sCD40L ng/ml**			
	Control (n=14)	1.44529	.136366	T=-5.823 P<0.001 Significant
	Study (n=16)	4.58937	2.011897	
2.	**S.CK-MB U/L**			
	Control (n=14)	15.29	4.531	T=-7.306 P<0.01 Significant
	Study (n=16)	53.13	18.871	
3.	**S.TC mg/dl**			
	Control (n=14)	165.50	18.430	T=-9.606 P<0.001 Significant
	Study (n=16)	256.56	30.956	
4.	**S.TGL mg/dl**			
	Control (n=14)	125.79	25.399	T=-6.175 P<0.001 Significant
	Study (n=16)	199.06	37.464	
5.	**S.HDL-C mg/dl**			
	Control (n=14)	44.14	9.223	T=3.890 P<0.05 Significant
	Study (n=16)	34.63	3.117	
6.	**S.LDL –C mg/dl**			
	Control (n=14)	96.93	19.329	T=-8.156 P<0.001 Significant
	Study (n=16)	183.25	35.179	
7.	**S.VLDL –C mg/dl**			
	Control (n=14)	25.14	5.082	T=-5.833 P<0.001 Significant
	Study (n=16)	38.69	7.264	
8.	**S.LDH U/L**			
	Control (n=14)	138.29	16.241	T=-2.641 P>0.05 Not Significant
	Study (n=16)	159.31	25.588	
9.	**S.AST U/L**			
	Control (n=14)	13.79	2.636	T=-3.185 P>0.05 Not Significant
	Study (n=16)	18.81	5.357	

TABLE 13: STATISTICAL ANLYSIS OF S. sCD40L, S.CK-MB, LIPID PARAMETERS AND S.LDH, S.AST IN CONTROL AND STUDY GROUP FOR ABOVE 60 YEARS OF AGE

SL.NO	VARIABLES	MEAN	S.D	STATISTICAL INFERENCE
1.	**S.sCD40L ng/ml**			
	Control (n=13)	1.44523	0.136242	T=-5.262 P<0.001 Significant
	Study (n=16)	4.79875	2.286681	
2.	**S.CK-MB U/L**			
	Control (n=13)	13.54	3.526	T=-6.482 P<0.01 Significant
	Study (n=16)	62.63	27.026	
3.	**S.TC mg/dl**			
	Control (n=13)	167.08	15.108	T=-6.549 P<0.001 Significant
	Study (n=16)	263.69	51.253	
4.	**S.TGL mg/dl**			
	Control (n=13)	125.23	28.856	T=-3.438 P<0.05 Significant
	Study (n=16)	179.56	50.578	
5.	**S.HDL-C mg/dl**			
	Control (n=13)	47.38	8.761	T=5.211 P<0.001 Significant
	Study (n=16)	34.31	4.453	
6.	**S.LDL –C mg/dl**			
	Control (n=13)	94.62	18.768	T=-7.129 P<0.001 Significant
	Study (n=16)	193.50	46.930	
7.	**S.VLDL –C mg/dl**			
	Control (n=13)	25.08	5.722	T=-3.404 P<0.05 Significant
	Study (n=16)	35.88	10.184	
8.	**S.LDH U/L**			
	Control (n=13)	136.92	8.261	T=-.414 P>0.05 Not Significant
	Study (n=16)	138.63	12.800	
9.	**S.AST U/L**			
	Control (n=13)	16.15	2.609	T=1.098 P>0.05 Not Significant
	Study (n=16)	15.00	2.966	

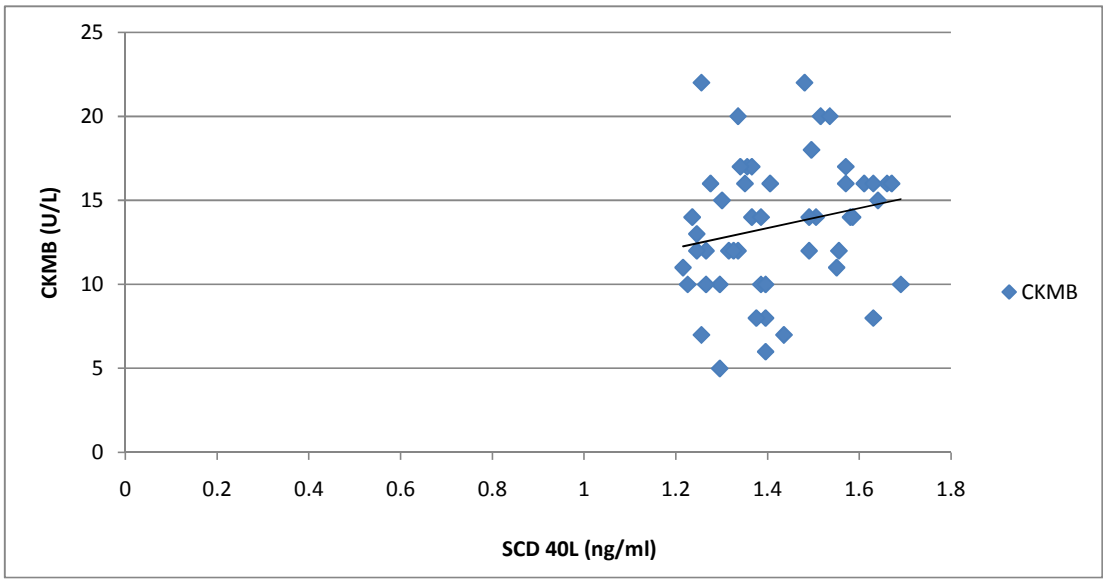
TABLE 14 : CORRELATION OF VARIABLES WITH DURATION OF CHEST PAIN IN THE STUDY GROUPS

SL.NO	VARIABLES	DURATION	MEAN	S.D	STATISTICAL INFERENCE
1.	S.sCD40L(ng/ml)	3Hours(n=11)	2.66500	1.820541	P<0.001 Significant
		4Hours((n=17)	4.19553	1.32895	
		5Hours(n=5)	5.90920	1.381516	
		6Hours(n=17)	5.27612	1.771021	
2.	S.CK-MB U/L	3Hours(n=11)	35.73	27.897	P<0.01 Significant
		4Hours(n=17)	58.71	17.211	
		5Hours(n=5)	76.80	13.442	
		6Hours(n=17)	60.53	16.938	
3.	S.LDH U/L	3Hours(n=11)	150.64	28.779	P>0.05 Not Significant
		4Hours(n=17)	153.71	24.057	
		5Hours(n=5)	141.80	12.518	
		6Hours(n=17)	144.94	18.397	
4.	S.AST U/L	3Hours(n=11)	17.82	5.288	P>0.05 Not Significant
		4Hours(n=17)	18.41	4.529	
		5 Hours(n=5)	14.40	2.074	
		6Hours(n=17)	15.94	4.548	

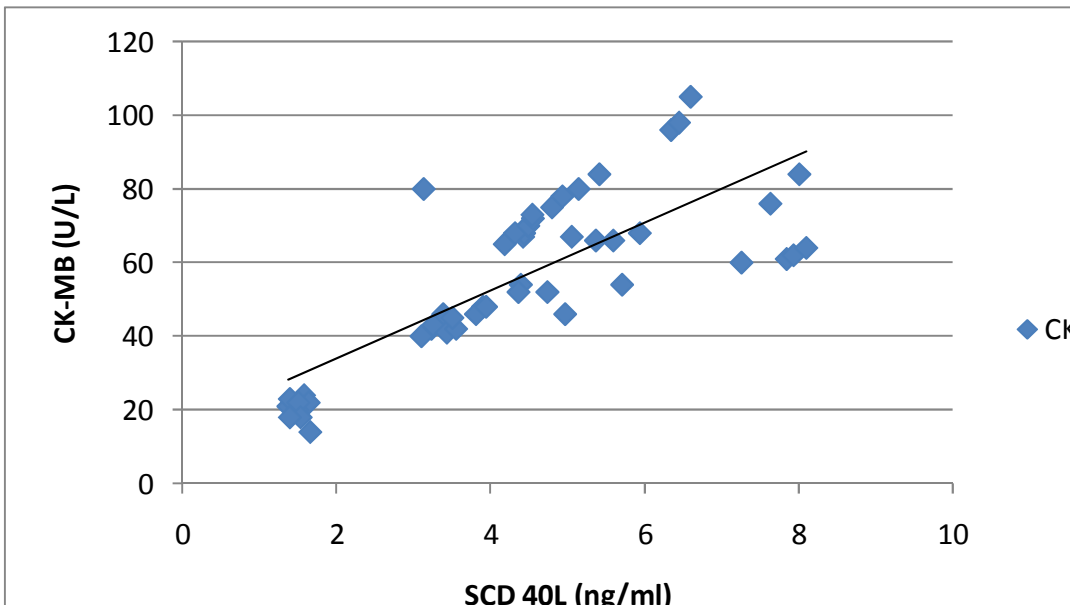
TABLE 15: PEARSON CORRELATION BETWEEN S. sCD40L AND OTHER BIOCHEMICAL PARAMETERS IN STUDY GROUPS

S.sCD40L ng/ml	CORRELATION VALUE	STATISTICAL INFERENCE
S.CK-MB U/L	0.777**	p<0.01 Significant
S.TC mg/dl	0.693**	p<0.01Significant
S.TGL mg/dl	0.596**	p<0.01 Significant
S.HDL mg/dl	-0.637**	p<0.01Significant
S.VLDL mg/dl	0.586*	p<0.01 Significant
S.LDL mg/ml	0.620**	p<0.01Significant
S.LDH U/L	-0.081	p>0.05Not Significant
S.AST U/L	-0.031	p>0.05 Not Significant

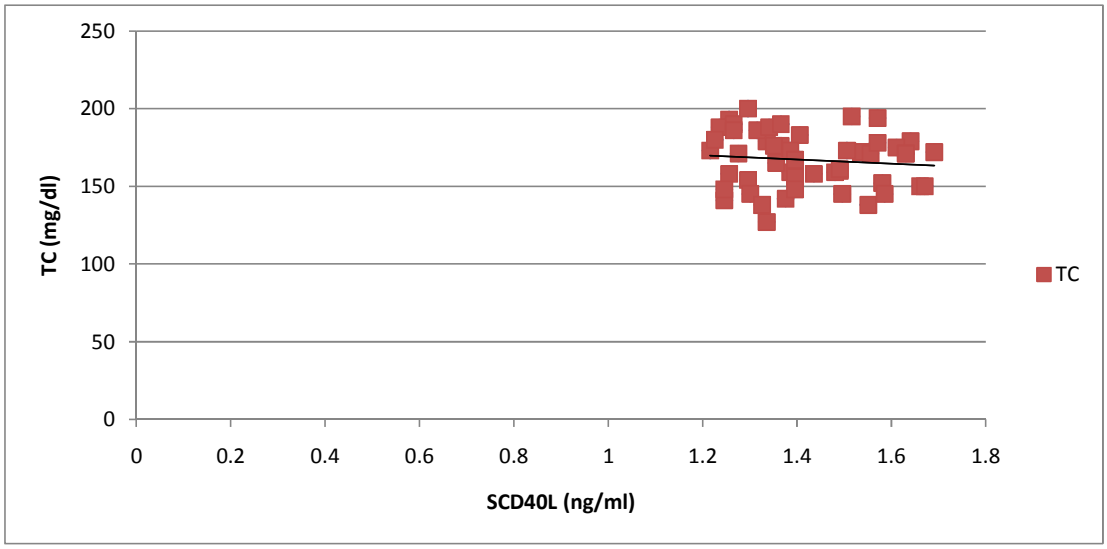
1. **SCATTER DIAGRAM OF S.sCD40L VS S. CK-MB IN CONTROLS**



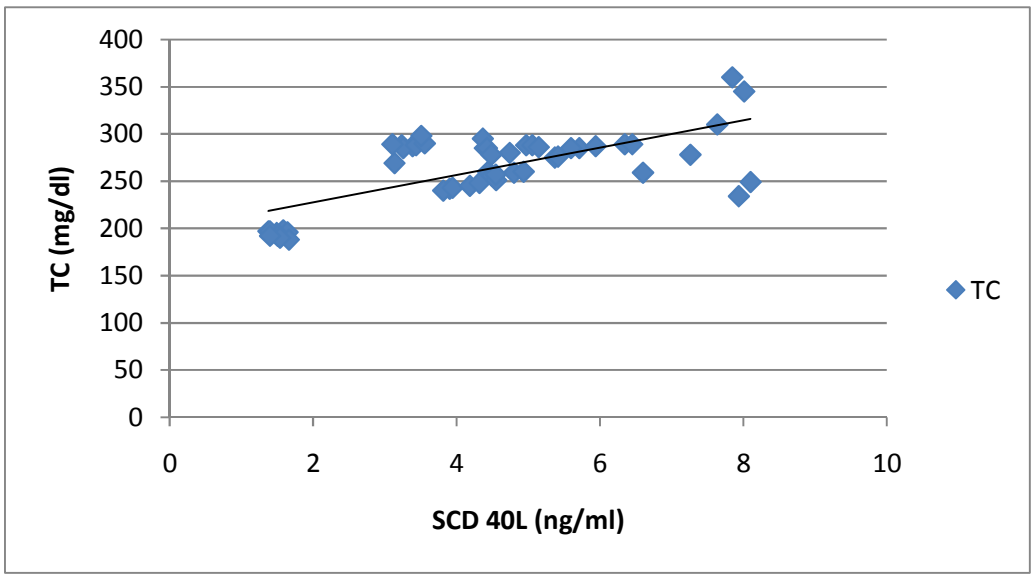
2.SCATTER DIAGRAM OF S.sCD40L VS S. CK-MB IN STUDY GROUPS



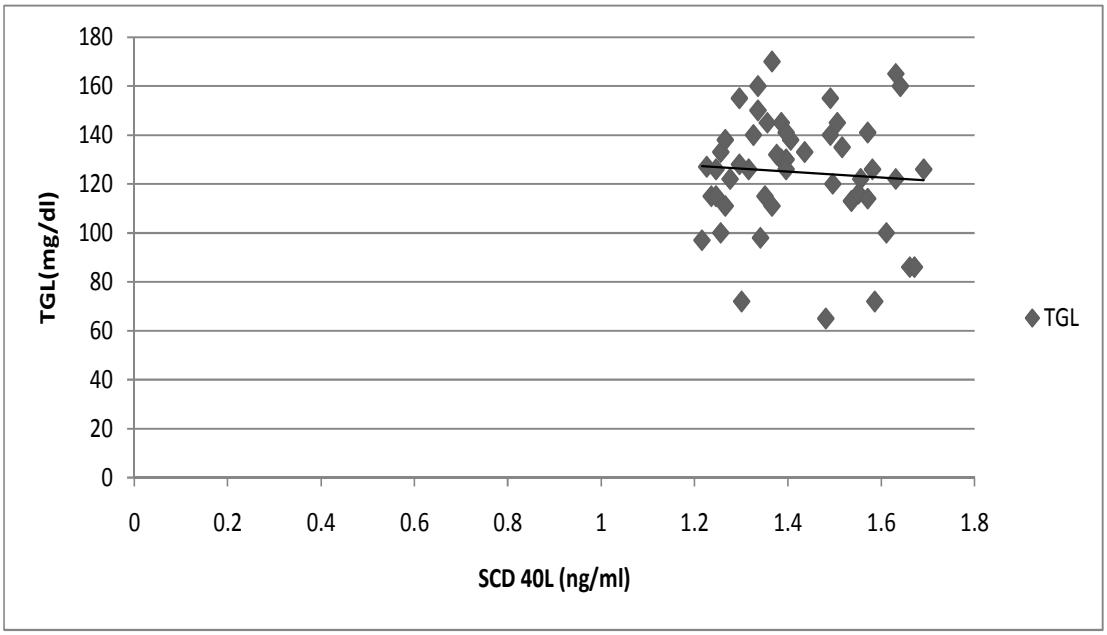
3SCATTER DIAGRAM OF S. sCD40L VS S. TC IN CONTROLS



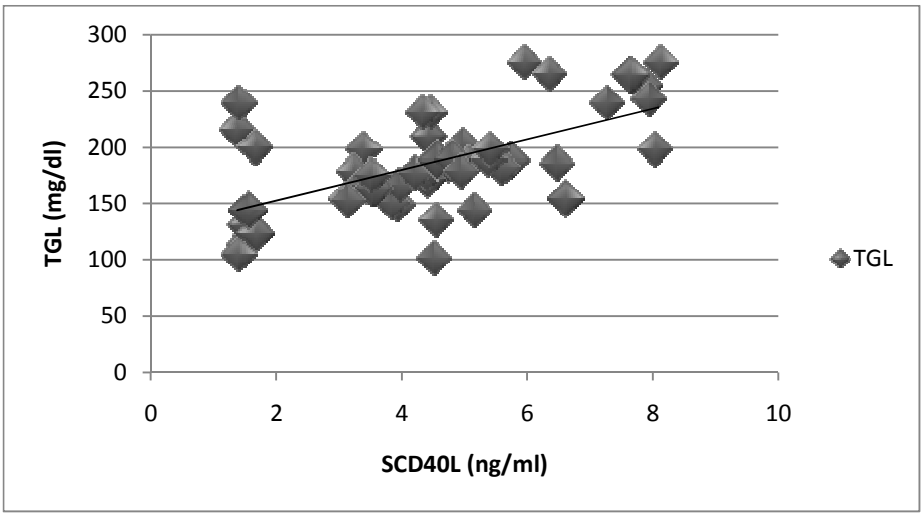
4 SCATTER DIAGRAM OF S. sCD40L VS S. TC IN STUDY GROUPS



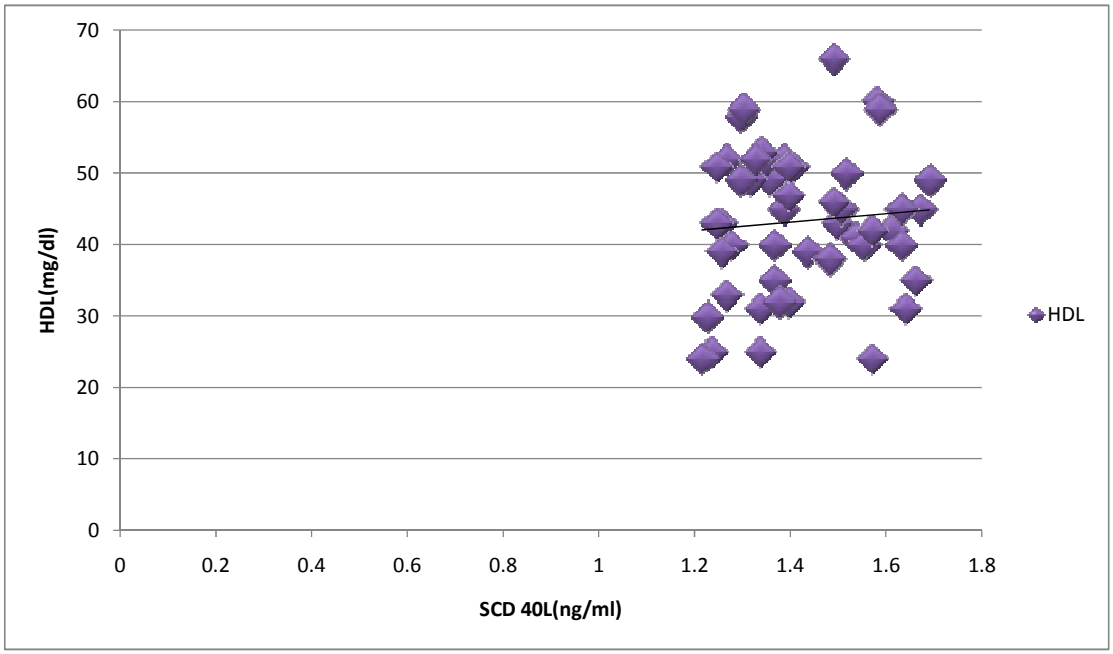
5.SCATTER DIAGRAM OF S.sCD40L VS S. TGL INCONTROL GROUPS



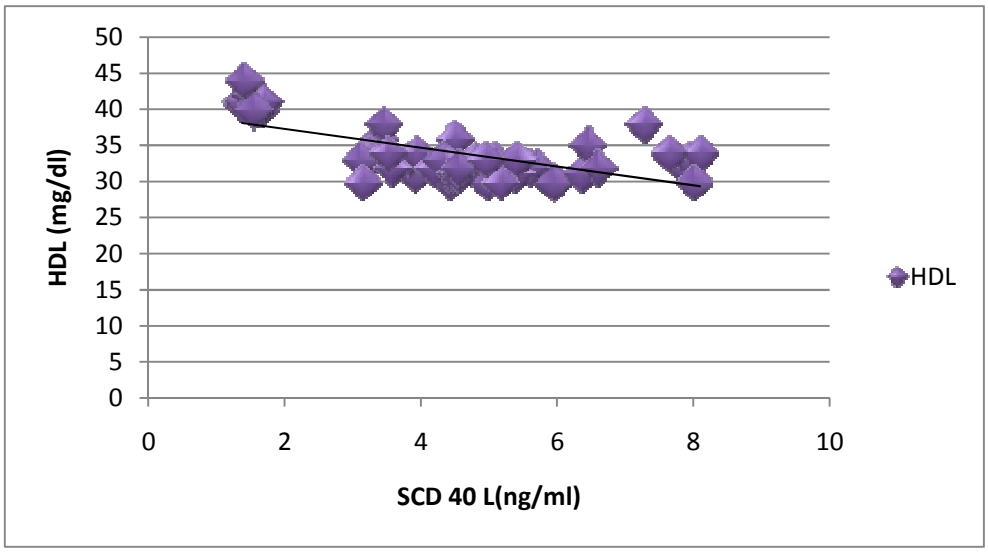
6.SCATTER DIAGRAM OF S. SCD40L VS S. TGL IN STUDY GROUPS



7. SCATTER DIAGRAM OF S.sCD40L VS S. HDL IN CONTROLS



8.SCATTER DIAGRAM OF S. sCD40L VS S. HDL IN STUDY GROUPS



RESULTS

A total of 100 subjects were included in the present study . Out of this 50 were under the study group (patients with ACS) and other 50 were under the control group, healthy individuals 20 females and 30 males were included in the study and control group. The values obtained in control and study group are presented in the master chart I&II respectively.

The serum levels of sCD40L and serum lipid parameters, fasting blood sugar , blood urea, serum creatinine, serum CK-MB, Serum LDH, Serum AST, S.VLDL, S.LDL-C levels were estimated in control and study group.

Table 1 shows the anthropometric features, the biochemical data , results of Serum sCD40L quantification, Serum CK-MB levels in the control and study groups. Serum sCD40L levels were in the range of 1.216 ng/ml to 1.691 ng/ml with a mean of 1.422 ng/ml in the control group.

Serum sCD40L levels in the study group ranged from 1.376 ng/ml to 8.099 ng/ml with a mean of 4.39758 ng/ml which is higher than the control group mean level.

Serum CK-MB in the control group ranged from 5 U/L to 22U/L with a mean of 13.48U/L . In the study group Serum CK-MB level ranged from 14 U/L to 105 U/L with a mean of 56.08 U/L which is higher than the control group mean level.

Table 2 shows age and sex distribution among study and control group subject (30 males and 20 females in each group) not significant in control groups (P=0.914>0.05) significant in study groups(P=0.035<0.05) .

Table 3 shows serum mean sCD40L levels in control and study group. Mean serum sCD40L level in study group is(4.397 ng/ml) which is higher than the control group mean of (1.422 ng/ml) which is statistically significant (P=0.0001<0.05)

Table 4 shows lipid parameters in study and control group there is a statistical elevation of S.TC, S.TGL, S.VLDL, S.LDL-C, in study group compared to control (P=0.0001<0.05) there is statistically significant decrease in the S.HDL-C levels in the study group compared to control group (P<0.001).

Table 5 shows mean Serum CK-MB values in study and control group. There is a statistically significant increase in the mean of study group (56.08± 22.58 U/L) compared to control group (13.48±4.047 U/L); (P <0.05).Compared to the values of Serum sCD40L the increase in values of Serum CK-MB does not show much significance.

Table 6 shows mean Serum sCD40L level in male control and study groups . There is a statistically significant increase in the mean of Serum sCD40L level in study group (4.569±1.670 ng/ml) compared to control group(1.442±0.151), (P=0.0001<0.05).

Table 7 shows mean Serum sCD40L level in female control and study groups. There is a statistically significant increase in the mean level of serum sCD40L in study group(4.139±2.227 ng/ml) compared to control group(1.392±0.117 ng/ml),($P=0.0001<0.05$).

Table 8 and 9 shows Serum sCD40L, Serum lipid parameters and Serum CK-MB levels in male and female groups respectively. Serum sCD40L, Serum lipid parameters and Serum CK-MB levels are high in study group compared to the control group both in males and females which is statistically significant.

Tables 10-13 shows age-wise statistical analysis of Serum sCD40L, Serum lipid parameters and Serum CK-MB, Serum LDH, Serum AST levels in control and study group.

Table 14 shows the statistical analysis of Serum sCD40L, Serum CK-MB, Serum LDH, Serum AST and duration of chest pain in the study group..

Table 15 shows Pearson's coefficient of correlation between Serum sCD40L and other biochemical parameters in study group.

There is a significant positive correlation between Serum sCD40L and Serum CK-MB, Serum TC, Serum TGL, Serum LDL-C, Serum VLDL and a negative correlation between Serum sCD40L and Serum HDL-C which is statistically significant. No significant correlation was found between Serum sCD40L with Serum LDH and Serum AST.

Bar Diagram I shows age and sex distribution of control and study groups..

Bar Diagram II shows mean Serum sCD40L levels in control and study groups.

Bar Diagram III shows Serum lipid parameters between control and study groups.

Bar Diagram IV shows mean Serum CK-MB levels in control and study groups.

Bar Diagram V shows mean Serum sCD40L level in male control and study groups.

Bar Diagram VI shows mean Serum sCD40L level in female control and study groups.

1. Scatter diagram of serum sCD40L vs Serum CK-MB levels in controls.

2. Scatter diagram of serum sCD40L vs Serum CK-MB levels in study groups.

3. Scatter diagram of serum sCD40L vs Serum TC levels in control groups.

4. Scatter diagram of serum sCD40L vs Serum TC levels in study groups.

5. Scatter diagram of serum sCD40L vs Serum TGL levels in control groups.

6. Scatter diagram of serum sCD40L vs Serum TGL levels in study groups.

7. Scatter diagram of serum sCD40L vs Serum HDL-C levels in control groups.

8. Scatter diagram of serum sCD40L vs Serum HDL-C levels in study groups.

DISCUSSION

The present study was done to evaluate levels of Serum sCD40L in patients with Acute Coronary Syndrome. It is a powerful biochemical inflammatory marker. .

In our study mean age of study group is (55.56±9.528 years) and that of control group is (52.22±9.412 years), mean BMI of study group and control group are equal and there is no statistical significance(25.295 versus 25.349, P>0.05).

In the present study mean serum sCD40L level of study group is higher than the control group (4.397±1.903 ng/ml versus 1.422±0.1395 ng/ml) which is statistically significant (P<0.05).This findings are in accordance with the study of Valerio sanguigni et al, (4.181±2.07 ng/ml in study group versus 2.60 ±0.7 ng/ml in control groups)[7].

In our study the mean level of serum sCD40L in ACS cases is (4.397±0.17 ng/ml) . This finding is almost consistent with Pal Aukurst et al study and Priya Gururajan et al study, reported higher levels of Serum sCD40L in ACS patients.

The mean Serum cholesterol level of (262.12±39.723 mg/dl) in the study group is higher than the control mean Serum cholesterol level of (166.98±17.757 mg/dl) which is statistically significant (P<0.05). This level is slightly higher than the mean level of Serum cholesterol observed in a study done by Priya Gururajan et al, and Mari Luomala et al[78].

The study group .mean Serum TGL level is (185.04±43.413 mg/dl) which is higher than the control group mean of 124.76±23.671 mg/dl which is statistically

significant($p<0.05$). Serum TGL level increases from 90 mg/dl to 180 mg/dl is associated with the doubled the incidence of CAD[79,80,81]

Mean Serum HDL cholesterol in the control and study group are (43.24±9.905mg/dl and 34.16±3.883 mg/dl) respectively. The mean serum HDL-C level in the study group is lower than the control group. It is statistically significant ($p<0.05$). Similar values are observed in Yogendra singh et al , study and demonstrated that low Serum HDL-C increases the risk of CAD..

The present study mean Serum LDL cholesterol of study group is higher than the control group (191.16±40.132 mg/dl versus 98.90±21.367 mg/dl) which is statistically significant($p<0.05$). Raised Serum LDL cholesterol has been recognized as a primary risk factor for CAD[81].

The mean level of Serum CK-MB value in the study group is higher than the mean level of Serum CK-MB in control groups (56.08±22.58 U/L versus 13.48±4.04 U/L).

The mean level of Serum sCD40L in males of the study group is higher than to the mean level of Serum sCD40L in males of the control group (4.569±1.670 ng/ml versus 1.442±0.151 ng/ml) which is statistically significant ($p<0.05$).

The mean level of Serum sCD40L in females of the study group is higher than to the mean level of Serum sCD40L in females of the control group (4.139±2.227 ng/ml versus 1.392±0.117 ng/ml) which is statistically significant ($p<0.05$).There is significance

difference in the mean values of lipid parameters, sCD40L and CK-MB in male and female groups.

Present study shows that there is significant increase in level of Serum sCD40L level in all age groups. Serum CK-MB shows not that much significance in comparison with Serum sCD40L levels.

Serum TC increase in all age groups (<40 years, 41-50 years, 51-60 years, 60 years). Serum TGL shows increased values in all age groups. Serum HDL-C does not show significant decrease under 40 years, but its values are significantly decreased in the age group of (41-50, 51-60, >60 years).

Serum LDL-C levels are also significantly raised in all age groups. VLDL-C values are not that much raised in <40 years but it is markedly elevated in remaining age groups. These variations can be attributed to the modifiable and non modifiable risk factors of atherosclerosis. Serum LDH and Serum AST values are not that much raised in all age groups.

There is a significant early rise in Serum sCD40L levels before Serum CK-MB, where as Serum LDH and Serum AST shows non significance

Pearson coefficient correlation analysis in the study group shows there is a highly significant positive correlation between Serum sCD40L and

S.CK-MB=($r=0.777$, $p<0.01$)

S.TC=(r=0.693, p<0.01)

S.TGL=(r=0.569, p<0.01)

There is negative correlation between Serum sCD40L and Serum HDL-C which is statistically significant (r=-0.637 p<0.01)[82.83].

Serum sCD40L contributes to atherosclerotic plaque destabilization and progression of chemokines, growth factors, cytokines and procoagulant factors in various cell types associated with atheroma. For thrombus formation, platelet activation is important, which in turn leads to precipitation of most of the unstable coronary syndromes.

Michelson AD et al found that large amounts of Serum sCD40L are produced and released from activated platelets. Yan et al in their study, demonstrated correlation between sCD40L and platelet activation. They also found that in patients with CAD, Serum sCD40L levels indicate an independent increased risk of major adverse cardiovascular events.

CONCLUSION

Biochemical markers such as Serum CK-MB, cardiac Troponin-I, and Myoglobin are used in the clinical setting to asses MI. However , elevation of these markers indicate myocardial necrosis and in the absence of necrosis these markers are not elevated . Serum sCD40L is a marker of inflammatory thrombotic activity that is expressed within seconds, after platelet activation.

Platelet activation and elevated fibrinogen level are associated with increased risk of Coronary thrombosis which is the gravest complication of atherosclerosis.

Since ACS is complicated by both myocardial necrosis and inflammation, assessment of both the processes may allow a better assessment of the disease.

Early intervention using anti-inflammatory drugs can be tried to prevent the progression of infarct size. This helps in reducing the morbidity and mortality from acute coronary syndrome.

LIMITATIONS OF THE STUDY

Serum sCD40L when coupled with coronary angiography would have aided in assessing the severity of coronary stenosis.

Analysis of genetic polymorphism in sCD40L would have enabled discrimination of various iso forms and their association with CAD.

FUTURE SCOPE OF THE STUDY

Serum sCD40L level estimation in outpatient department itself can be studied in patients complaining of chest pain to rule out myocardial ischemia before ECG manifestation.

It can be used to rule out non anginal causes of chest pain.

BIBLIOGRAPHY

1. Park.k.Epidemiology of Chronic Non-Communicable Diseases and Conditions. In: Park.k, editor. Parks text book of preventive social medicine. 20th edition.Jabalbur, India;.M/s Banarsidas Bhanot pub; 2009. p.15-317.
2. Bhaskara Rao. Prevention of Non- Communicable Diseases.In: Baskara Rao, editer. Text book of Community Medicine;2nd edition. Hyderabad; Paras Medical Pub;2006.p.162-163.
3. Michael C. Kim/Annapoorna S. Kini/ Valentin Fuster. Definitions of Acute Coronary Syndromes. In: Valentin Fuster, Robert A.O Rourke, Richard A.Walsh, et al, editors. Hurts The Heart.12th edition. New York: Mc Graw Hill medical pub; 2008. p. 1311-1312,1316-1318
4. Elliot M.Antman, Andre P. Selwyn, Eugene Braunwald, Joseph Loscalzo. Ischemic Heart Disease.In:Anthony S.Fauci,Dennis L.Kasper,Dan L.Longo, et al,editors. Harrisons Principles of Internal Medicine.volume II.17th edition.NewYork. Mc Graw Hill pub; 2008. p. 1524.
5. J.C.E Underwood. Cardiovascular System.In:J.C.E Underwood, editor. General and Systemic Pathology.4th edition. Edinburgh: Churchill Livingstone pub;2004. p. 280, 301.
6. Harsh Mohan. The Blood Vessels and Lymphatics.In: Harsh Mohan, editor. Text Book of Pathology.6th edition.NewDelhi: Jaypee brothers Medical pub;2010. p. 393-395.
7. Ahmed H. Kissebah, Glenn R. Krakoner. Clinical Manifestations of the Metabolic Syndrome.In:Geroge A.Bray, Claude Bouchard,W.P.T.James, editors. Hand Book of Obesity.NewYork: Marcel Dekker Inc;1998.p. 604
8. Christian W. Hamm,Christopher Heeschen, Erlind Falk and Keith A.A. Fox. Acute Coronary Syndromes.In:A.John Camm, Thomas F.Luscher Patrick W.Serruys,editors.The ESC Textbook of Cardiovascular Medicine.1st edition.Massachusetts: Blackwell pub;2006. p. 336
9. D.E. Newby N.R. Grubb, A.Bradbury. Cardiovascular Disease.In:Nicki R.Colledge,Brian R.Walker,Stuart H.Ralston,editors. Davidsons Principles and

Practice of Medicine.21st edition.Philadelphia: Churchill Livingstone Elsevier pub;2010. p. 577-578.

10. Roberta J. Emerson,Naomi Lungstrom. Alterations in Blood Flow. In: Lee-Ellen C.Copstead, Jacquelyn L.Banasik,editors. Pathophysiology Biological and Behavioral Perspectives.2nd edition.Philadelphia:W.B.SAUNDERS COMPANY pub;2000. p.365
11. Gorden A.A.Ferns, William J. Marshall and Stephen K. Bangert. The clinical biochemistry of the cardiovascular system.In: William J.Marshall, Stephen K.Bangert, editors. Clinical Biochemistry Metabolic and clinical aspects.2nd edition.Philadelphia: Churchill Livingstone Elsevier pub;2008. p. 786-789
12. Russell V. Luepher. Heart diseaseIn:Robert B.Wallace,Neal Kohatsu,John M.Last, et al,editors.Wallace/Maxcy-Rosenau-Last Public health& preventive medicine.15th edi.NewYork:Mc Graw Hill Medical pub;2008. p. 1080-1081
13. Dr.Michael Clark.Ischemic Heart Disease. In: Professor ParveenKumar,Dr Michael Clark,editors.Kumar & ClarksClinical Medicine.7th edition.Philadelphia: Saunders Elsevier pub;2009. p. 747.
14. Frederick J.Schoen,Richard N.Mitchell.The Heart.In: Vinay Kumar,Abul K.Abbas, Nelson Fausto,et al ,editors. Robbins & Cotran pathologic basis of disease.8th edition.Philadelphia: Saunders Elsevier; 2010.p. 503-504,546
15. Marc Hartmann ,Clemens von Birgelen, Gary S.Mintz, et al,editors. Relation Between Lipoprotein(a) and Fibrinogen and Serial Intravascular Ultrasound PlaqueProgressioninLeftMainCoronaryArteries;JournaloftheAmericanCollege of Cardiology;Vol.48,No.3, 2006: 446-452.
16. Andrew D. Michaels. Coronary Heart Disease.In:Thomas E.Andreoli, Ivor J.Benjamin, Robert C.Griggs, et al ,editors.Andreoli and Carpenters Cecil Essentials of Medicine.8th edition.Philadelphia: Saunders Elsevier pub;2011.p. 97
17. .Dev B.Pahlajani. ST- Elevation Myocardial Infarction.In:Siddarth N.Shah,M.Paul Anand,Aspi R.Billimoria, et al,editors. API Textbook of Medicine.8th edition.Mumbai; The Association of Physicians of India, Mumbai pub; p. 520

18. Patricia Revest. The Cardiovascular System.In:Jeannette Naish,Patricia Revest,Denise Syndercombe Court,editors. Medical Sciences.1st edition.Philadelphia: Saunders Elsevier pub ;2009. p. 634
19. Mihai Gheorghide Robert O.Bonow Coronary Artery Disease. In: H.David Humes, Herbert L.Dupont,Laurance B.Gardner, et al,editors. Kelleys Textbook of Internal Medicine.4th edition.Philadelphia: Lippincott Williams and Wilkins pub;2000.p. 446
20. J.B.Walter.Ischemia and Infarction. In:J.B.Walter,I.C.Talbot, H.Allen Gardner, ,et al,editors.Walter and Israel General Pathology.7th edition. Philadelphia: Churchill Livingstone Elsevier pub;2009. p. 715-716
21. Luigi Giusto Spagnoli, Elena Bonanno,Giuseppe Sagiorgi,et al. Role of Inflammation in Atherosclerosis. Journal of Nuclear Medicine; nov 2007: vol 48,No 11 , 1800-1815.
22. Judd E. Hollander ,Deborah B. Diercks. Acute Coronary Syndromes: Acute Myocardial Infarction and Unstable Angina.In:Judith E.Tintinalli, J.Stephen Stapczynski,David M.Cline,O.John Ma,et al,editors. Tintinallis Emergency Medicine.7th edition.NewYork: Mc Graw Hill medical pub;2011. p.367
23. Eugene Braunwald. Clinical Recognition of Acute Coronary Syndromes.In: Pierre Theroux,editor. Acute Coronary Syndromes.2nd edition.Philadelphia: Saunders Elsevier;2011.p.95-96
24. C D Garlichs,S, Eskafi,D.Raaz, et al. Patients with acute coronary syndromes express enhanced CD40ligand /CD154 on platelets.Heart.2001;86: 649-655.
25. Alan Stevens. Arterial Diseases.In:Alan Stevens,James Lowe,Ian Scott,,editors. Core Pathology.3rd edition.Missouri: Mosby Elsevier pub;2009.p. 174
26. Christopher P.Cannon and Eugene Braunwald. Unstable Angina &Non ST Elevation Myocardial Infarction.In: Robert O.Bonow,Douglas L.Mann,Douglas P.Zipes, et al,editors. Braunwalds Heart disease. 9th edition.Philadelphia: Saunders Elsevier;2012. p.178-1179
27. Valendina L. Brashers.Alterations of Cardio Vascular functions.In:Sue E.Huether,Kathryn L.McCance,Valentina L.Brashers et al,editors.

Understanding Pathophysiology..4th edition.Missouri:Mosby Elsevier pub;2004. p. 631

28. .Palazzuoli Alberto, Iovine Francesca,Scali Chiara et al.Acute Coronary Syndromes: From The Laboratory Markers To The Coronary Vessels. Biomarkers insights. 2006: 1, 123-130
29. Noriaki Kume, Hirokazu Mitsuoka, Kazutaka Hayashida Masaru Tanaka,et al. Soluble Lectin-Like Oxidized Low-Density Lipoprotein Receptor -1 Predicts Prognosis After Acute Coronary Syndrome. Circulation Journal.2010; May 14 .
30. Thuy Anh Nguyen,Jean G. Diodati,Chantal Pharand Pharm D. Resistance to Clopidogrel: A Review of the Evidence.Journal of the American College of Cardiology.2005;Vol.45, No. 8.
31. D.M.Vasudevan.Cardiovascular Diseases and Hyperlipidemias.In:DM .Vasudevan,Sreekumari .S,Kannan Vaidyanathan,editors.Text Book of Biochemistry For Medical Students.6th edition.NewDelhi:Jaypee Brothers Medical pub;2011.p. 292
32. Erling Falk, Pradimen K. Shah, Valentin Fuster. Coronary Plaque Disruption. Circulation; 1995; 92: 657-671.
33. Avrum I. Gotlieb.Blood Vessels.In:Raphael Rubin,David S.Strayer,Emanuel Rubin,et al, editors. Rubins Pathology.5th edition.Philadelphia: Wolters Kluwer / Lippincott Williams & Wilkins; 2008. p. 399-400
34. Earl P. Benndittt and Stephan M. Schwartz. Blood Vessels.In:Emanuel Rupin,John L.Farber,editors. Pathology.Philadelphia: J.B. Lippincott Company pub;1988. p. 462-463.
35. Eric H. Awtry, Joseph Loscalzo. Coronary Heart Disease.In:Thomas E.Andreoli,Charles C.J.Carpenter,Robert C.Griggs,et al ,editors. Cecils Essentials of Medicine.6th edition.Philadelphia:Saunders Elsevier pub; 2005.p. 87
36. Michael J.Davies Coronary Disease. The pathophysiology of acute coronary syndromes. Hear.2000; 83: 361-366.

37. C.G. Bahuleyan. Ischemic Heart DiseaseIn:KV KRISHNA Das,editor. Textbook of Medicine.5th edition.NewDelhi:JAYPEE BROTHERS MEDICAL PUBLISHERS(P) LTD;2008. p. 844
38. S.C.Manchanda.Ischemic Heart Disease.In:S.C.Manchanda,editor.Orams clinical Heart Disease.3rd edition.NewDelhi:CBS Publishers and Distributors;1999. p. 287-288
39. Ming-Chao Lin,Fanny Almus –Jacobs,HsuanHsu Chen, et al . Shear Stress Induction of the Tissue Factor Gene. J. Clin. Invest.1997; Volume 99,Number 4: February 737-744.
40. Rene R.S. Packard and Peter Libby. Inflammation in Atherosclerosis: From Vascular Biology to Biomarker Discovery and Risk Prediction. Clinical Chemistry. 2008;vol.54 no.1 :24-28.
41. David A.Vorchheimer, Richard Becker. Platelets in Atherothrombosis. Mayo Clinic Proceedings. January 2006; vol.81 no.1: 59-68.
42. Drik Lievens, Alma Zernecke,Tom Seijkens,et al.Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. Blood. November 18,2010; vol.116 no.20:4317-4327.
43. Carmen Urbich, Ziad Mallat,Alain Tedgui, et al. Upregulation of TRAF -3 by shear stress blocksCD-40 mediated endothelial activation. The Journal of Clinical Investigation. November 2001;volume 108/ number 10:p. 1451-1458
44. Amy H.Kao, Janice M. Sabatine, Susan Manzi.Update on vascular disease in systemic lupus erythematosus. Current Opinion in Rheumatology. 2003; 15: 519-527.
45. Giorgio Baroldi. Diseases of the Coronary Arteries.In:Malcolm D.Silver,editor. Cardiovascular Pathology .volume 2.NewYork: Churchill Livingstone Inc;1983. p. 331-333.
46. Ron Waksman, Pauline E .McEwan,Travis I.Moore, et al. Photo Point Photodynamic Therapy Promotes Stabilization of Atherosclerotic Plaques and Inhibits Plaque Progression. Journal of the American College of Cardiology.2008;Vol.52,No.12:1024-1032.

47. Charalambos Antoniades, Constantinos Bakogiannis,Dimitris Tousoulis,et al.Linking Inflammation With Atherotrombosis. The CD40/CD40Ligand System, State of the art paper accepted. March 10,2009.

48. Aurelie S. Leroyer, Pierre-Emmanuel Rautou,Jean –Sebastien Silvestre, et al.CD40Ligand+Microparticles From Human Atherosclerotic Plaques Stimulate Endothelial Proliferation and Angiogenesis. Journal of the American College of Cardiology.2008;52:1302-11.

49. Mihail Hristov, Denis G umbel,Esther Lutgens, et al. Soluble CD40Ligand Impairs the Function of Peripheral Blood Angiogenic Outgrowth Cells and Increases Neointimal Formation After Arterial Injury. Circulation. 2010; 121: 315-324.

50. Hoong Sern Lim, Andrew D. Blann,Gregory Y.H.Lip. Soluble CD40 Ligand, Soluble P-Selectin, Interleukin-6,and Tissue Factor in Diabetes Mellitus.Circulation.2004;109:2524-2528.

51. Uwe Schonbeck , Peter Libby.CD40 Signalling and Plaque Instability.,Circulation Research. 2001;89: 1092-1103.

52. Valerio Sanguigni, Domenico Ferro,Pasquale Pignatelli, et al.CD40 Ligand Enhances Monocyte Tissue Factor Expression and Thrombin Generation Via Oxidative Stress in Patients With Hypercholesterolemia. Journal of the American College of Cardiology.2005; vol 45,No.1, :35-42.

53. Carmen Urbich, Elisabeth Dernberg,Alexandre Aicher, et al.CD40 Ligand Inhibits Endothelial Cell Migration by Increasing Production of Endothelial Reactive Oxygen Species. Circulation ,Journal of the American Heart Association. 2002;106:981-986.

54. .Saif Anwaruddin and Eric J.Topol. Inflammation Status.In:Eric J.Dopol,editors. Textbook of Interventional Cardiology.5th edition.Philadelphia:Saunders Elsevier pub;2009. p.4-5

55. Paik-Seong Lim,Ming-ying WU,Shiaw-Wen Chein,et al. Elevated circulating levels of soluble CD40ligand in haemo dialysis patients with symptomatic coronary heart disease. Nephrology .2008;13:677-683.

56. Allan S. Jaffe, Lciano Babuin,Fred S.Apple. Biomarkers in Acute Cardiac Disease. Journal of the American College of Cardiology.2006;Vol.48,No.1:1-11.

57. Christopher Heeschen, Stefaine Dimmeler,Christian W.Hamm,et al. Soluble CD40Ligand in Acute Coronary Syndromes. N Eng J Med. 2003;348.1104-11.

58. Pal Aukurst , Fredrik Muller,Thor Ueland, et al. Enhanced Levels of Soluble and Membrane –Bound CD 40 Ligand in Patients With Unstable Angina: Possible Reflection of T Lymphocyte Platelet Involvement in the Pathogenesis of Reflection of Acute Coronary Syndromes. Journal of the American Heart Association. Circulation;1999; 100;614-620.

59. C D Garlichsa, S Eskafia,D Raaza, et al. Patients with acute coronary syndromes express enhanced CD40ligand/CD154 on platelets. Heart. 2001; 86: 649-655.

60. Alexander Hermann, Bernhard H. Rauch, Marina Braun, et al. Platelet CD40ligand (CD40L) –subcellular localization, regulation of expression, inhibition by clopidogrel .Platelets. 2001;12, 74-82.

61. Drik Lievens, Alma Zernecke,Tom Seijkens, et al. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis.BLOOD.18 November 2010 Volume 116, Number 20.

62. Boris T. Ivandic, Eberhard Spanuth,Detlef Haase, et al. Increased plasma concentrations of Soluble CD40 Ligand in Acute Coronary Syndrome Depend on in vitro Platelet Activation. Clinical Chemistry. july 2007,vol.53 no.7,000-000(2007) 1231-1234.

63. Francesca Santilli ,Giovanni Davi,Agostino Consoli, et al. Thromboxane Dependent CD40Ligand Release in Type 2 Diabetes Mellitus.Journal of American College of Cardiology.2006; Vol .47, No . 2.

64. Thor Ureland, Pal Aukurst, Arne Yndestad, et al.Soluble CD 40 Ligand in acute and chronic failure. European Heart Journal.2005; 26 ,1101-1107.

65. C.M.Nagesh& Ambuj Roy. Role of biomarkers in risk stratification of acute coronary syndrome .Indian J Med Res November2010;132,pp627-633.

66. Priya Gururajan ,Prema Gurumurthy,Pradeep Nayar, et al.Increased Serum Concentrations of Soluble CD40 Ligand As a Prognostic Marker in Patients with Acute Coronary Syndrome. Indian Journal of Clinical Biochemistry. 2009/24(3) 229-233.

67. Halil Genc,Teoman Dogru,Serkan Tapan,et al. Soluble CD40 ligand , soluble P-selectin and von Willebrand factor levels in subjects with prediabetes: The impact of metabolic syndrome.Clinical Biochemistry. 45 (2012) 92-95.

68. Fred S.Apple, Alan H.B.Wu, Johannes Mair, et al. Future Biomarkers for Detection of Ischemia and Risk Stratification in Acute Coronary Syndrome. Clinical Chemistry.2005; 51:5 810-824.

69. Nerea Varo,David Vicent, Peter Libby, et al. Elevated Plasma Levels of the Atherogenic Mediator Soluble CD40 Ligand in Diabetic Patients: A Novel Target of Thiazolidinediones. Journal Of The American Association, Circulation published.May12,2003.

70. Zienat A El Kholy, Neveen A Hussein,Usama A Sharaf El-Din, et al. Effect of Low –Molecular Weight Iron Dextran Therapy on Pregnancy Associated Plasma Protein –A and CD40 Ligand Markers in Chronic Renal Failure Patients. Journal of the Medical Research Institute.2009;vol,30 no.2;(105-110

71. IIkay Tugba Unek,MD, Firat Bayraktar,Dilek Solmaz, et al.The Levels of Soluble CD40 Ligand and C-Reactive Protein in Normal Weight, Overweight

and Obese People. Clinical Medicine & Research .2010;Volume 8,Number 2: 89-95.

72. Lisa Nannizzi –Alaimo, Mark H .Rubenstein,Veronica L.Alves, et al. Cardiopulmonary Bypass Induces Release of Soluble CD40 Ligand. Journal of American Heart Association, Circulation.2002; 105: 2849-2854.

73. By Melanie K. Spriggs, Richard J. Armitage,Laura Strockbine, et al. Recombinant Human CD40Ligand Stimulates B Cell Proliferation and Immunoglobulin E Secretion. J.Exp.Med.December1992; volume176:543-1550.

74. Cees van Kooten and Jacques Banchereau. CD40-CD40 ligand. Journal of Leukocyte Biology.January2000; Volume 67.

75. By BLAIR . Renshaw, William C. Fanslow, et al. Humoral Immune Responses in CD40 Ligand –deficient Mice. J.Exp.Med.November1994;volume 180 november 1994 1889-1900.

76. Dimitris Daoussis, Andrew P. Andonopoulosand Stamatis-Nick C.Liossis. Targeting CD 40 : a Promising Therapeutic Approach. Clinical Diagn Lab Immunology. 2004, 11(4): 635. 635-641.

77. Valerio Sanguigni, Pasquale Pignatelli,Luisa Lenti, et al. Coronary Heart Disease , Short Term Treatment With Atorvstatin Reduces Platelet CD40 Ligand and Thrombin Generation in Hypercholesterolemic Patients. Circulation.2005; 111: 412-419.

78. Mari Luomala, Hannu Paiva,Reijo Laaksonen,et al.Plasma –soluble CD40 is related to cholesterol metabolism in patients with moderate hypercholesterolemia. Scandinavian Cardiovascular Journal. 2006 ; 40: 280-284.

79. HS Rissam S. Kishore, N Trehan. Coronary Artery Disease in Young Indians-The Missing Link. Journal, Indian Academy of Clinical Medicine.September 2001; Vol .2,No .3,p128-131.

80. Nader Rifai,G.Russel Warnick, Lipids, lipoproteins, apolipoproteins and other cardio vascular risk factors , Teitz text book of clinical chemistry and molecular diagnostics, edited by Carl A.Burtis, Edward R. Ashwood, David E.Bruns,4th edition,2006,Saunders an imprint of Elsevier, pages: 924,1623-1629,1633.

81. Yogendra Singh, Saurabh Srivastava,Sohabib Ahmad, et al. Is Lipid Tetrad Index the Strongest Predictor of Premature Coronary Artery Disease in North India ?. JIACM. 2010; 11(3) :175-9.

82. Garlichs CD , John S. Schmeisser A,Eskaki S.Stumpf, et al . Upregulation of CD40 and CD40 Ligand (CD154) in Patients with moderate hypercholesterolemia.Circulation.2001 Nov 13; 104 (20) :2395-400.

83. Francesco Cipollone, Andrea Mezzetti, Ettore Porreca, et al. Association Between Enhanced Soluble CD40Land Prothrombotic State in Hypercholesterolemia: Effects of Statin Therapy.Circulation.2002; 106: 399-402.

A STUDY OF SERUM SOLUBLE CD40 LIGAND LEVEL IN PATIENTS WITH ACUTE CORONARY SYNDROME – PROFORMA

O.P/I.P.NO:

Name of the patient :

Age/sex :

Occupation :

Address :

Complaints :

Past history :

Personal history :

Family history :

Drug history :

General examination : Ht: (mts) Wt: (kg) BMI: (kg/mt^2)

BP: (mmHg) Pulse rate: /minute

Specific examination : CVS: RS:

ABD: CNS:

Investigations:

1. Blood sugar (fasting): mg/dl

2. Blood urea: mg/dl

3. Serum creatinine: mg/dl

4. Serum soluble CD40 Ligand: ng/ml

5. Lipid profile:

S. Total cholesterol: mg/dl

S. Triglycerides: mg/dl

S. HDL-C: mg/dl

S.VLDL-C: mg/dl

S. LDL-C: mg/dl

6. Serum CK-MB: U/L

7. Serum LDH: U/L

8. Serum AST: U/L

CONSENT FORM

Dr.M.Manonmani post graduate student in the department of Biochemistry, Thanjavur Medical College, Thanjavur is doing A Study of Serum Soluble CD40 Ligand Level in Patients With Acute Coronary Syndrome. The procedures has been explained to me clearly. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature:

Name:

Place: